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# Multidimensional Mass Spectrometry of Chemonic™ CCG-6 Nonionic Surfactant With Separation by Polarity and Shape

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*Multidimensional Mass Spectrometry of Chemonic™ CCG-6  
Nonionic Surfactant  
With Separation by Polarity and Shape*

**Honors Research Project**

Submitted to

*The University of Akron Williams Honors College*

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## Preface

### *Mass Spectrometry of Poly(vinyl Alcohol) Crosslinking with Tetraborate Ions using Atmospheric Solids Analysis Probe*

It must be mentioned that the University of Akron Williams Honors college received a formal proposal from me in October of 2018 about my intention to work with poly(vinyl alcohol) (PVA) to characterize the crosslinking that occurs when tetraborate ion is introduced into the system. I proposed that I would use the atmospheric solids analysis probe (ASAP) as an ionization source with a Synapt mass spectrometer to verify the character of the crosslinking that occurs. This project was inspired by an ACS article about “slime” science that had corrected a prior article after readers corrected some very common misconceptions about this system. The goal was to use ASAP as a simple ionization method without much prep to verify the lesser-known crosslinking mechanism discussed in the ACS article, and secondarily to use this project as an introduction to mass spectrometry principles and methods.

After weeks of attempted experiments, results were inconclusive. Previous work showed that tetraborate ionizes in negative ion mode. However, when attempted in lab, no negative ions were observed with any ASAP settings. In positive ion mode, distributions of  $14.02n$  Da were analyzed. PVA's repeat unit weight is 44.05 Da, so distributions of  $44.05n$  Da were expected. Likely, the ASAP source is too strong of an ionization source or the hot nitrogen gas required of the instrument is too high energy and causes decomposition of the product in-source, rendering results unlike the expected system. For this reason, my research leader, Jason O'Neill, recommended that I shift my focus on a new project that has shown more success. The new project parallels previous work with multidimensional spectrometry of complex mixtures of nonionic surfactants. This new project has provided much more conclusive data that taught me complex analytical techniques and data analysis. Due to the late project change and higher level of complexity of the new project, I will report more 'fundamental' analysis.

## Abstract

Chemonics™ CCG-6 surfactant is a water-soluble poly(ethylene glycol) (PEG) conjugated alkyl glyceride emollient. This surfactant exists as a complex mixture of a glycerol cores conjugated with poly(ethylene glycol) branches (PEGylation) that were partially esterified with caprylic (C8) and capric (C10) acids. These may be esterified on one, two, or all three arms of the glyceride. The architecture of the structures in this mixture was studied using multidimensional mass spectrometry (MS). Mass spectrometry was interfaced with ultra-performance liquid chromatography (UPLC) and ion mobility (IM) separation. The mixture was separated by reversed-phase LC, oligomers of the star-branched polymer were separated according to their relative polarity, denoted by different retention times. While LC separated according to polarity, IM spectrometry isolated the oligomers of the system by their collision cross-sectional area and charge or size/shape. In either separation method, increasing the fatty acid ester content increased the drift time (DT) or size/shape, and retention time (RT) or polarity. Rudimentary characterization of the mixture identified a range of PEGylated mono-, di-, and triacylglycerides as well as the same moieties attached as acyldiglycerides to a much lesser extent. Over 80% of the compounds are identified as having one or two fatty acid groups, and the overall split of caprylic to capric acids is near 55%/46% C8/C10. The average degree of PEGylation is about 5 units of PEG per molecule type. LC-MS and IM-MS, when used in conjunction, complement each other to separate isobaric species.

## Executive Summary

### *Problem Statement*

Mass spectrometry is a sensitive analytical technique for the study of gas-phase ions. By acquiring the mass-to-charge ratio ( $m/z$ ) of these ions, any fragment ions formed during the ionization steps, these  $m/z$  values are used to deduce the composition and structure of the analyte. However, MS is hampered by the following limitations: MS does not reveal specific information about functional groups present in a polymeric material or about its primary and higher-order structure, nor can mixtures and blends be characterized properly due to differences in ionization and detection efficiencies of their constituents. For this reason, physical separation methods have been coupled with MS. Commonly, reverse-phase liquid chromatography or gas chromatography is performed before MS. In this case, LC separated by relative polarity of components. For even more complex mixtures, further separations in series can be employed before MS. Ion mobility separates ions by relative collisional cross section (CCS). By coupling these two methods of separation, the various components in the product can be accurately characterized. A nonionic surfactant such as Chemonics™ CCG-6 is a polymeric product that is also a blend of different isomers that would be difficult to appropriately characterize with MS alone. CCG-6 was chosen to explore the method due to its mix of oligomers with varying amounts of fatty acids and degrees of PEGylation.

### *Results and Conclusions*

By assuming that all compounds in the surfactant ionize with the same relative ease, one may use the relative intensities of distributions from mass spectra. Using this, the sample was characterized as 70.8% mono-, di-, and triacylglycerides. 18.8% of the sample is 'lone' PEGylated fatty acids that aren't reacted with glyceride chains, and the balance 10.4% represents mono-, di-, and triacyldiglycerides, in which glycerols form ethers during the polymerization reaction. Of all the glycerides and diglycerides, 43.5% and 38.5% represent one and two fatty acid chain ends. The relatively low amount of triacylglycerides is verified by the 18.8% of 'lone' PEGylated fatty acid chains detected. Of the whole sample, 56.3% of compounds with fatty acids contain C8, while 43.7% contain C10. The relative degree of

PEGylation can be estimated by selecting the  $m/z$  with the greatest intensity. In the whole sample, an average  $n = 5$  with a  $\text{stdev} = 1.3$  PEG units. In particular, the larger the molecule, the greater likelihood of PEGylation. This is seen when glycerides have an average  $n = 5$  with  $\text{stdev} = 0.8$  PEG units but diglycerides (which have 4 bonding locations over glycerides that have 3 bonding sites) have an average  $n = 7$  with  $\text{stdev} = 0.4$  PEG units. A less important, but interesting enough, detail to present is that competitive adduct formation occurred in the sample. The larger chemical structures preferred ammoniation while smaller ions preferred sodiation. This difference is likely due to the relative sizes of sodium and ammonium ions.

### *Conclusions and Recommendations*

The goal of the analysis is to develop and verify a method for multidimensional spectrometry, which is a success. The accomplishment is emphasized by the elementary characterization of the mixture. The multidimensional separation uncovered acyldiglyceride compounds that were not initially expected in any appreciable amount, which demonstrates the power of this methodology. Similar in-line separation techniques should be considered in an industrial setting for quality control or for formulation tuning. The procedure should be repeated for other complex materials, such as paints, oils, or other processing additives that exist as complex mixtures.

### *Project Implications*

Learning analytical chemistry techniques and principles may seem outside the scope of an undergraduate engineer's academic career, but I dispute that every engineer should understand the fundamentals of analytical chemistry. This is especially useful if the engineer operates in research, processing, or quality control, because engineers need to understand deviations and material properties of their products. Therefore, I chose a research project in analytical chemistry with a focus on polymeric materials. CCG-6 is not only a system of oligomers but is an example of an application of complex mixtures – a surfactant. By partaking in this project, I was able to learn elementary surfactant science as well as analytical chemistry principals, methods, instrumentation, and data analysis techniques. I was pushed to understand

proposed ionization mechanisms and adduct formation then apply all the aforementioned to a polymeric surfactant system to execute elementary analysis.

### *Future Work*

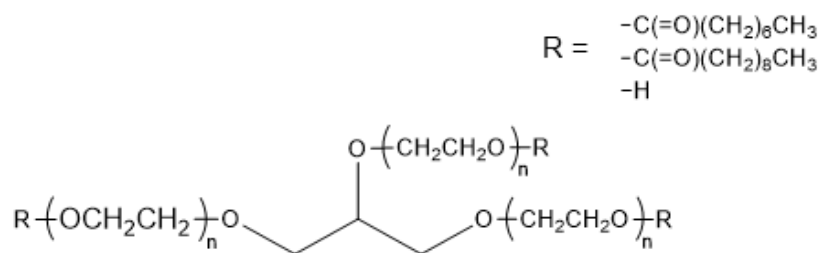
Looking forward, I would be interested to see how this multidimensional separation could be implemented to study complex natural systems (i.e. *Hevea* rubber, *Guayule* rubber, etc.). Both plants contain natural rubber to a minor extent and resinous components to a major extent, such as triglycerides, terpenes, fatty acids, proteins, and even components that have potential for medicinal applications. With such a complex mixture, it is difficult to understand relative amounts and purification methods without understanding what is truly in the system. Using multidimensional analysis, more light can be shed on such biological resources. From my fledgling experience in the natural rubber industry, there is still a lot to learn about natural rubber sources. I urge any chemist, biochemist, or chemical engineering student to consider analytical chemistry projects for research because the tools they learn will carry over into almost any field they follow. All research and development positions, whether in synthesis, separations, compounding or formulation all require strong analytical testing to verify their metrics, and to have a strong understanding of these techniques will allow someone to communicate results and goals with ease.



## Introduction and Background

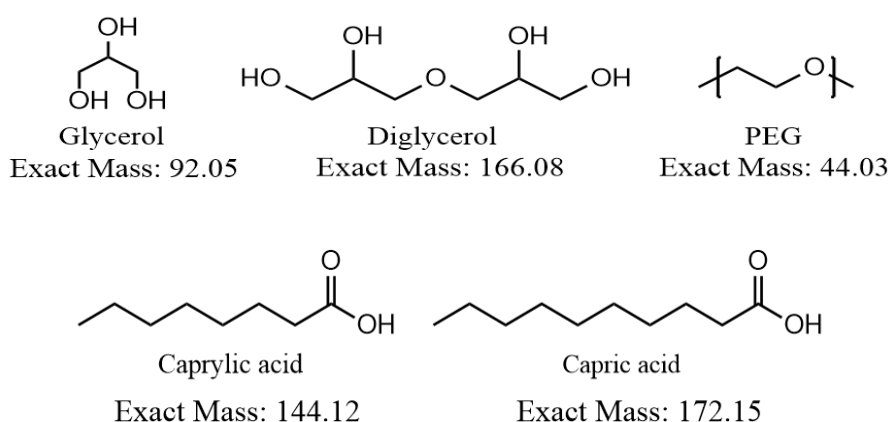
The primary focus of this project is to characterize a nonionic surfactant mixture. Surfactants are substances that adsorb onto surfaces in a system and alter the interfacial free energy of those respective surfaces. Most surfactants are amphiphilic organic compounds, meaning they contain both hydrophobic (relatively nonpolar) groups and hydrophilic (relatively polar) groups. Therefore, surfactants contain both water-soluble and water-insoluble components. These compounds diffuse into solvents and adsorb at interfaces between different fluid phases, altering the surface tension at this interface. The most common example of this is soap allowing the interfacial tension between water and oil (immiscible in water) to decrease and allow the oil to effectively dissolve in water.

Chemonic™ CCG-6 surfactant is a water-soluble poly(ethylene glycol) (PEG) conjugated alkyl glyceride emollient and emulsifying agent added to skin cleansing products for clarity and cohesion of other components (i.e. such as natural oils) in water-based formulations. PEGylated alkyl glycerides are mono-, di-, and/or triglycerides that have been modified with poly(ethylene glycol) (PEG). A specific molar equivalence of ethylene glycol repeat units are combined with mono- or diglyceride blends of C8/C10 fatty acids under heat and pressure with an alkaline catalyst until < 1 ppm of ethylene oxide remains unreacted [1]. Two reactions occur; etherification of the free alcohol groups on the glycerol and glycerides with ethylene oxide groups and transesterification of the PEG groups between glycerol and fatty acid components of the glyceride [1]. To a lesser extent, glycerol cores may react to form a diglycerol (see *Figure 1*). Such reactions result in a glycerol or diglycerol core with three chains of PEG groups with one, two, or all three of the PEG chains terminated by a fatty acid.



*Figure 1* - Structure for PEG-6 Caprylic/Capric Glycerides.

Many PEGylated alkyl surfactants used in cosmetics are named in the form of ABC-X (i.e. CCG-6). In this paper, where the value of X in PEG-X is equal to 6 (e.g., PEG-6 caprylic/capric glycerides), X represents the number of stoichiometric equivalents of ethylene oxide that were added to one stoichiometric equivalent of caprylic/capric glycerides [1]. Also, when denoting amount of PEG in surfactants of this type, the representing structures are PEG<sub>n</sub>, where n = degree of polymerization. Therefore, the sum of all the different n PEG values on each branch of the glycerides in the mixture may not be more than X. A percentage of the ethylene oxide simply polymerizes with itself and remains unattached to any glycerol.



*Figure 2* - Primary components of the nonionic surfactant mixtures in Chemonic™ CCG-6

Due to reactions between the groups in *Figure 2*, PEGylated alkyl glycerides are blends of homologous structures containing other species such as lone PEG chains, fatty acids or glycerol moieties. This complex formulation requires novel methods to separate and elucidate structures. High-performance liquid chromatography (HPLC) has been used to separate components of surfactants before [4], but traditional LC detectors (i.e. UV-vis, refractive index, and evaporative light scattering) do not provide the precision required to understand these complex blends. Fortunately, the more robust detectors of mass spectrometry can be beneficial. Thus, the complementary features of MS detectors can be used by interfacing LC with MS to study specific compositions of complex molecules [2, 3, 9].

Electrospray Ionization (ESI) is a versatile, sensitive, and reliable soft ionization technique for use with MS that can be interfaced with LC. Soft ionization refers to ionization techniques that are less likely to cause fragmentation in-source. The advent of soft ionization techniques (e.g. ESI) inspired applications of MS to proteins, synthetic polymers, and non-covalent complexes. In positive ion mode, the number of charged species normally observed in an electrospray spectrum is reflected in the number of basic sites on a molecule that can be protonated at low pH [5, 6]. By interfacing LC, ESI allows for complicated tandem separations in series. A more novel development is Ion Mobility Spectrometry (IMS). It has grown popular in the pharmaceutical industry for its ability to separate proteins by their conformations due to the nature of the separation. The molecules are propagated by an electric field against an inert gas, where the collisional cross section of the analyte relates to the speed at which the molecule travels. [8, 10, 11]. Thus, the following experiment utilizes UPLC-ESI-IM-MS for multidimensional separation and analysis of PEGylated glyceride surfactant.

## Materials and Procedure

### *Materials*

Chemonic™ CCG-6 surfactant, which is supplied as “PEG-6 Caprylic/Capric Glycerides” was provided by Lubrizol Advanced Materials, Inc (Cleveland, OH). HPLC grade methanol and water were purchased from Sigma-Aldrich (St. Louis, MO). All materials were used in the condition received from their supplier.

### *Liquid Chromatography*

The reversed-phase LC separation was performed on a Waters Acquity UPLC system (Waters Corporation, Milford, MA, USA) using a Phenomenex Luna Omega C18 100 Å column (100 x 2.1 mm, 1.6 µm particle size) held at 50°C. The run time was 30 minutes with a flow rate of 200 µL/min. Two mobile phases were employed. Mobile phase A consisted of H<sub>2</sub>O with 0.1% formic acid. Mobile phase B consisted of ACN with 0.1% formic acid. A post-column T-inlet was used for addition of 2.5 mM NH<sub>4</sub>(CH<sub>3</sub>COO) in 1:1 (v:v) ACN:H<sub>2</sub>O at 100µL/min to promote ammoniation. Gradient elution was performed as follows:

1. Initial - A: 60%, B: 40%
2. 8 min - A: 40%, B: 60%
3. 9 min - A:30%, B: 70%
4. 20 min - A:5%, B: 95%
5. 30 min - A:5%, B: 95%

### *Mass Spectrometry*

A 20 mg/mL solution was made by dissolving 20 mg of CCG-6 in 1 mL of 1:1 (v:v) H<sub>2</sub>O:MeOH. The sample was then diluted to 200 ppm (mg/kg) in 7:3 (v:v) H<sub>2</sub>O:MeOH and mixed, then filtered using an Acrodisc Polyvinylidene Fluoride (PVDF) syringe filter (13 mm, 0.2 µm) (Pall Corp, Port Washington, NY) into an LC-MS vial. UPLC-ESI-IM-MS experiments were performed by interfacing the Acquity UPLC system with a Synapt HDMS quadrupole/time-of-flight (Q/TOF) mass spectrometer (Waters Corporation, Milford, MA). The sample solution was introduced to

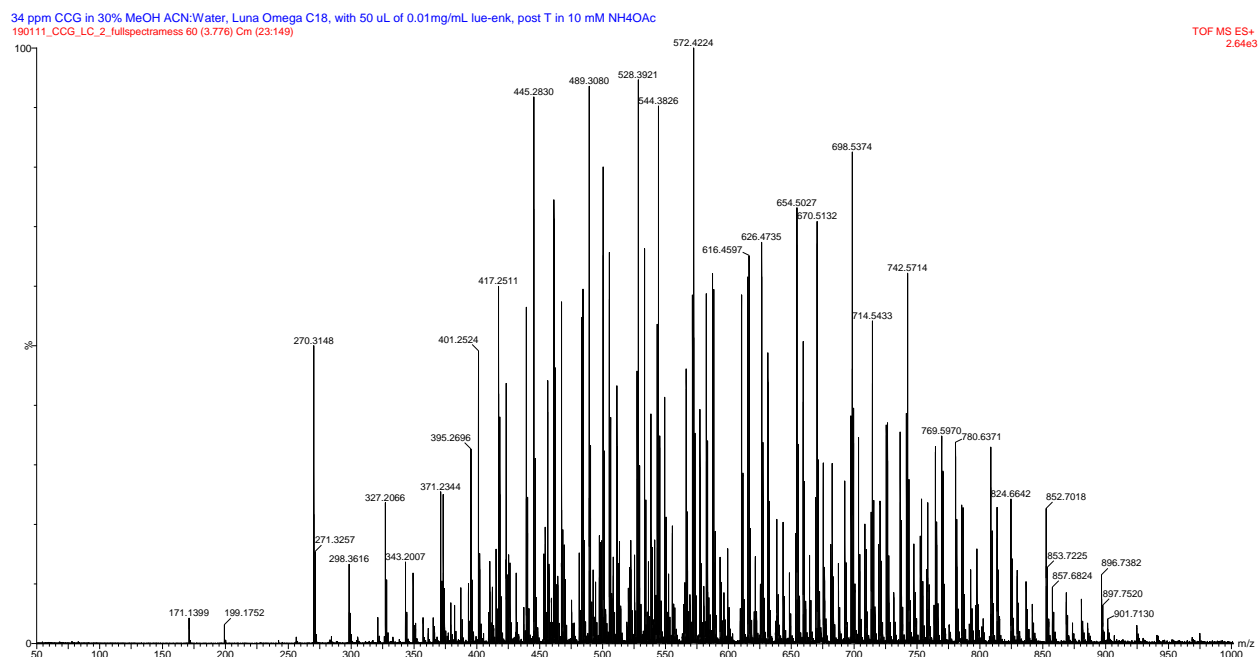
the ESI source by direct infusion. The instrument was operated in positive ion mode with a capillary voltage of 3.1 kV, cone voltage of 35.0 V, source temperature of 100 °C, and desolvation gas flow rate of 600 L/hr at 250 °C. After samples pass the ESI source and ions move through the quadrupole, ions enter the triwave region - comprised of the trap, ion mobility, and transfer cells. The trap cell collects and directs ions to the IM chamber, where the ions are propagated by low voltage waves against a stream of nitrogen gas. The IM separation was achieved by tuning the source traveling-wave height and velocity in the IM cell to 8.0 V and 300 m/s and setting the nitrogen flow rate to 22.70 mL/min.

### *Safety*

Due to the nature of chemical labs, safety measures must be taken to minimize risk when performing experiments. Such actions always include the use of proper PPE such as gloves and safety eyewear. Many of the chemicals being dealt with in the laboratory are flammable and volatile. Therefore, it is important to keep a clean work environment and perform work with chemicals under a fume hood. At the end of dealing with chemicals it is important to dispose of them properly. Chemical wastes in Dr. Wesdemiotis' lab is to be poured into the appropriate satellite waste receptacle, then the chemical's previous container placed under the specified waste hood evaporate residual solvents. After that time the vials or test tubes can be disposed or cleaned. If a spill is to occur, the area should be cleared, and the lab should be ventilated if the spill is significant. Another concern in the lab is the handling of glass vials and syringes. If any glassware breaks, one is to immediately clean the glassware up using a broom and dustpan. Glass waste containers are located throughout the lab and all glass waste should be disposed there. Cleaning broken glassware should never be done without safety eyewear and gloves. Finally, the mass spectrometer instrumentation is very precise and made to detect very small concentrations of samples. Therefore, cleaning is often required before and after running samples to completely clean the instrumentation.

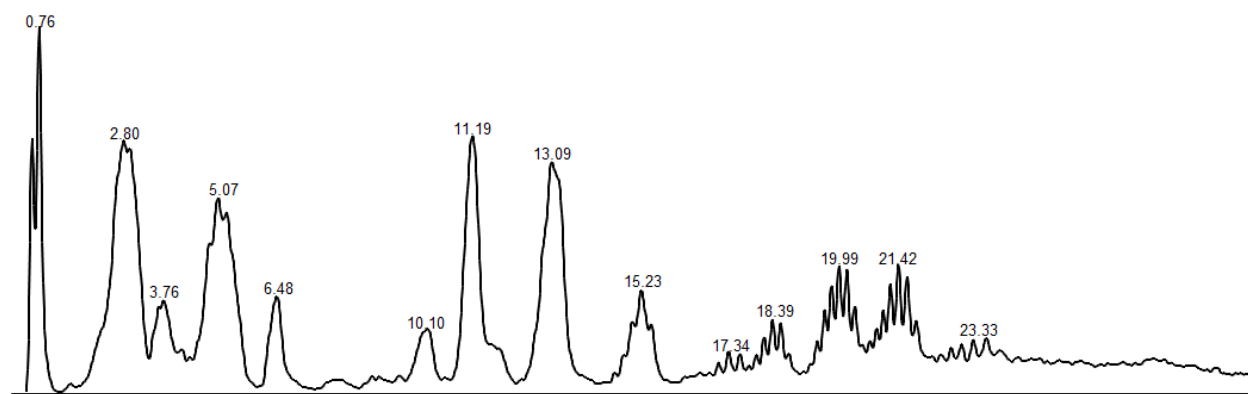
## Results and Discussion

One should never expect a surfactant to be a single, pure compound, because surfactants are complex mixtures of oligomers. This should be expected by understanding how this mixture was synthesized. Partial etherification of PEG onto glycerol as well as transesterification of fatty acids to terminate the PEG chain ends means that there will be constituents left unreacted. [1] Prior to LC-MS or IM-MS analysis, direct injection MS was performed. As can be seen below (Figure 3), a combined mass spectrum of the surfactant shows a multitude of ions with few distinguishable characteristics or patterns. It would be difficult to determine how many compounds are in this sample, but there are some notable distributions with peaks such as  $572.42 \pm 44.05n$  Da (G1PEGC10 + NH<sub>4</sub>),  $626.47 \pm 44.05n$  Da (G1PEGC8C8 + NH<sub>4</sub>), or  $654.48 \pm 44.05n$  Da (G1PEGC10C8). It is expected that there are significantly more structures in the sample, but they are not able to be revealed. The aforementioned ions are the most notable because they result in the suppression of ionization of other expected structures, either due to their ease of ionization or because they are present in the greatest amounts. Thus, it is a necessity for physical separation methods such as LC and IM to isolate the different structures in this sample. In the case of CCG-6, two major determinants of the surfactant properties are the number of ethylene glycol units and the number of fatty acid moieties. The balance between these components affects the activity of the surfactant. Controlling the distribution of these components is key to maintaining quality control of a surfactant product. By using separation methods that capitalize on the differences that arise from varying amounts of PEG or acids, we can learn more about the system.



*Figure 3 – Combined spectrum from direct injection MS.*

Initial analysis of the reverse-phase LC-MS data in *Figure 4* shows there is 4 possible groupings of molecules separated by polarity. Intuitively, increasing fatty acid character and decreasing glycerol and PEG units increases the retention time (RT). Using this knowledge, we can estimate the constituents of each grouping. At RT of 0.76 minutes, we expect the relatively polar unreacted glycerol cores and PEG chains to pass through the polar stationary phase quickly. Previous work by Borisov reported that the most hydrophilic species in polysorbates elute this quickly in LC-MS [9]. From RT of 2.8-6.5 minutes; the PEGylated monoglycerides, from RT of roughly 10.1-15.2 min; PEGylated diglycerides, and the remaining eluent is PEGylated triglycerides. Even with this level of separation, there are likely other anomers that are not noticed. Look at the peak of eluent with retention time of 2.8 minutes from *Figure 4*. Due to its early retention, one expects it to have the least fatty acid character, likely a C8-monofacylglyceride. There is a very slight shelf to the polar side of the peak in the distribution that leads investigators to believe there is more here. Further separation with IM could answer this.



*Figure 4 - Total Ion Current (TIC) from UPLC-MS.*

The above estimates are verified and corrected once post-ionization IM separation is amended to the analysis. By adding the second separation technique, one may see that there are peaks with similar RTs but significantly different DTs, components that would not be seen without IMS. Looking again at the example eluent with a RT of 2.8 minutes, now with DT representing relative size, there is clearly two compounds eluting at nearly the same time. The upper structure, with the greater size, is a diglycerol core with a single PEGylated fatty acid. This was not initially considered as a part of the sample until IM was used. The diglycerol core has more hydroxyl groups and thus more bonding sights for PEG. For this reason, acyldiglyceride constituents of the surfactant are more polar but larger, with generally lower RTs and higher DTs than their acylglyceride counterparts. In *Figure 5*, the power of multidimensional separation can be seen. Moving forward, the focus shifts to verifying groups 2 through 4 (PEGylated mono-, di-, and triacylglycerides and acyldiglycerides as well as free PEGylated fatty acids). *Figure 6* displays the TIC annotated by grouping.



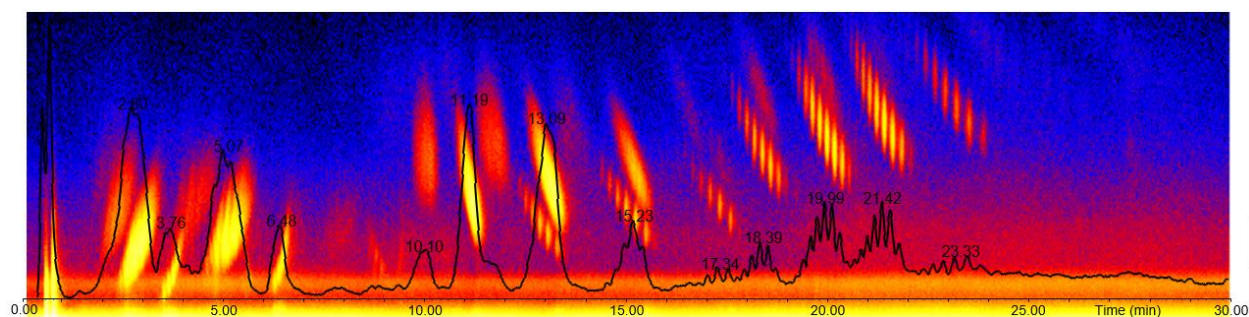


Figure 5 - Overlay of TIC from UPLC-MS on TIC from UPLC-IMS-MS with retention time (polarity) on the abscissa and drift time (size and shape) on the ordinate.

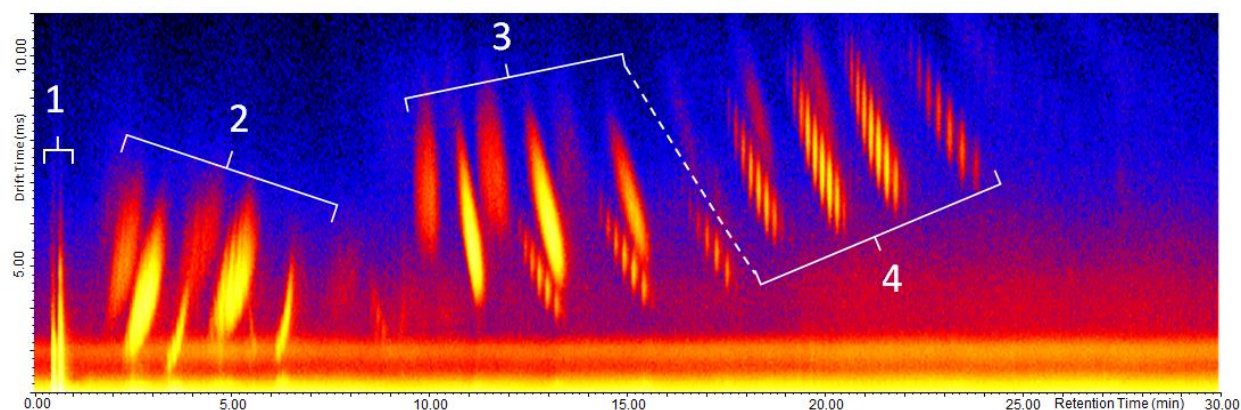


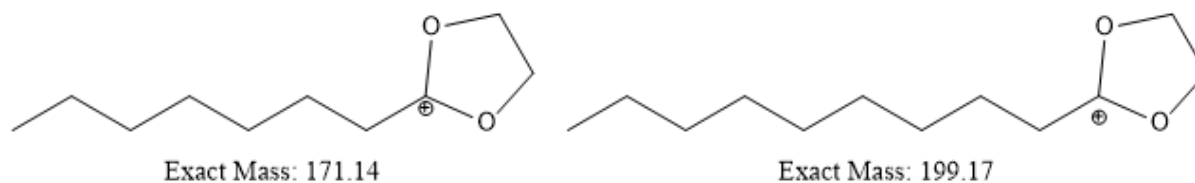
Figure 6 - Groupings of related structures of PEG-6 Caprylic/Capric Glycerides.

Table 1 - Summary of related groupings of structures from PEG-6 Caprylic/Capric Glycerides.

Group	Constituents
1	Unesterified PEG-glycerols and free PEG chains.
2	PEGylated monoacylglycerides or monoacyldiglycerides of C8 or C10 as well as free PEGylated fatty acids.
3	PEGylated diacylglycerides or diacyldiglycerides of C8-C8, C10-C8, or C10-C10 as well as free PEGylated fatty acids.
4	PEGylated triacylmonoglycerides of C8-C8-C8, C10-C8-C8, C10-C10-C8, or C10-C10-C10.

A useful indicator to verify the presence of a specific C8 or C10 acid moiety is the presence of fragmentation peaks in the form of dioxolane carbocations.  $M/z$  values of 171.14 and 199.17 Da for C8 and C10, respectively, are present in the mass spectra that contain C8 and/or C10.

Despite the conditions and soft ionization of ESI, these fragments occur in the source. See *Figure 7* for these structures.



*Figure 7* - C8 and C10 fragment dioxolane carbocations distinct to this system that allow us to designate the existence of unattached fatty acids.

In group 2; RT of 2.8 to 5.5 mins contains PEGylated caprylic and capric monoacyldiglycerides, monoacylglycerides as well as free PEGylated acids. There appear to be 3 pairs of associated peaks with DTs. The greatest DT pairs must have the greatest collisional cross-section area, representing diglycerol cores. The decreasing DT or 'size,' are as follows: the diglycerol core monoacyldiglycerols, the single glycerol monoacylglycerols, and then unattached PEGylated fatty acids. In increasing RT (decreasing polarity), these pairs should represent C8 acid and then C10 acid constituents.

In group 3; RT of 10.1 to 17.3 min should follow in decreasing polarity by increasing acid content from group 2. Therefore, these peaks should represent PEGylated diglycerides with two fatty acids attached to the end of the cores. There seem to be 3 groups (in descending DT or 'size') that represent the diglycerol cores, glycerol cores, and free PEGylated acids. In increasing RT, these should represent all combinations of C8 and C10 acids; C8-C8 diglycerides, C10-C8 diglycerides, then C10-C10 diglycerides.

In group 4; RT of 18.4 to 23.3 min, no diglycerol cores nor free fatty acids seem to exist in any appreciable amount. This group is predominantly PEGylated triglycerides characterized, in increasing RT, as C8-C8-C8, C10-C8-C8, C10-C10-C8, and C10-C10-C10.

Mass spectral analysis verifies all the above. Ion mobility and MS analyses were performed with Driftscope™ 2.0 and MassLynx™ (Waters Corporation, Milford, MA, USA), respectively. As a

basis, it was assumed that every peak of the TIC contained structures with 6 PEG units, then the MS peaks with the greatest relative intensities were marked as the actual amount of PEG units attached to each compound. The intensities of signals from MS were used to estimate relative amounts of the different structures in the samples for a simple mass balance. Assuming all components ionize with the same relative frequency is necessary to make these calculations. These are only rough estimates, and the amounts are summarized in *Table 2* and *Table 3*.

Most of the surfactant mixture is about 71% single glycerol core structures, 19% free PEGylated acids, and 10% diglycerol core structures. A total mass balance between the two types of fatty acids in the system show about 56% C8 and 44% C10. This seems reasonable, since the Cosmetic Ingredient Review reports that the surfactant is blended with 60% C8 and 40% C10 fatty acids [1]. The majority of all acylglycerides and acyldiglycerides contain one or two fatty acids, comprising 82% of the mixture. An overall count of the average amount of bonded PEG unit shows about 5 PEG units per component of the mixture. This makes sense, because the product is made by adding 6 stoichiometric equivalents of PEG, and it is reasonable to assume that not all 6 PEG equivalencies esterified with glycerol cores.

*Table 2* - Summary of identified structures from PEG-6 Caprylic/Capric Glycerides. M represents the mass of the proposed structure with 6 PEG units, even though the sample's most common number of PEG units may deviate from this value.

ID	DT	RT	M "PEG6"	[M+NH4] <sup>+</sup>	[M+NH4] <sup>+</sup> (obs)	ppm error	avg PEG	Rel. Amount
PEGC8	2.11	3.74	364.25	382.28	382.28	-10.5	5	7%
G1PEGC8	3.26	2.94	482.31	500.34	500.35	12.0	5	13%
G2PEGC8	4.29	2.36	556.35	574.38	574.43	80.1	8	3%
PEGC10	2.24	6.51	392.28	410.31	410.33	39.0	4	4%
G1PEGC10	3.58	5.07	510.34	528.37	528.39	30.3	5	15%
G2PEGC10	4.93	3.99	584.38	602.41	602.46	76.4	7	2%
PEGC8C8	3.71	12.85	534.38	552.41	552.45	65.2	4	3%
G1PEGC8C8	4.74	11.19	608.41	626.44	626.47	41.5	6	10%
G2PEGC8C8	5.76	10.11	682.45	700.48	700.54	79.9	7	3%
PEGC10C8	4.29	15.23	562.41	580.44	580.49	79.2	4	3%
G1PEGC10C8	5.06	13.17	636.44	654.47	654.5	39.7	6	11%
G2PEGC10C8	6.46	11.64	710.48	728.51	728.58	90.6	7	3%
PEGC10C10	4.48	17.32	590.44	608.47	608.53	92.0	4	2%
G1PEGC10C10	5.7	15.32	664.48	682.51	682.56	67.4	4	4%
G1PEGC8C8C8	5.95	18.6	734.52	752.55	752.61	74.4	4	4%
G1PEGC10C8C8	6.34	19.99	762.55	780.58	780.64	71.7	4	6%
G1PEGC10C10C8	6.98	21.42	790.58	808.61	808.68	81.6	4	6%
G1PEGC10C10C10	7.3	23.22	818.61	836.64	836.74	114.7	4	2%

*Table 3* - Summary of the relative amounts of PEG attached to each type of structure.

	avg (PEG units)	stdev (PEG units)	amount
<b>Lone Chains</b>	4	0.4	18.8%
<b>Glycerol Core</b>	5	0.8	70.8%
<b>Diglycerol Core</b>	7	0.4	10.4%
<b>C8 Acids</b>	6	1.4	56.3%
<b>C10 Acids</b>	5	1.1	43.7%
<b>1 Fatty Acid</b>	6	1.4	43.6%
<b>2 Fatty Acids</b>	5	1.3	38.5%
<b>3 Fatty Acids</b>	4	0	17.9%
<b>Overall</b>	5	1.3	

To promote single ionization adducts, ammonium acetate is introduced post-UPLC. This system singly ionized, as distinguished by the isotope patterns between each peak equal to 1 Da. For the sake of brevity, any m/v value is equivalent to its M value and will be referred to as such unless specified otherwise. Sodium ions are also inevitably present on glassware, on the source,

and in the instrumentation. Therefore, competitive distributions of  $[M+Na]^+ \pm 44.03n$  Da and  $[M+NH_4]^+ \pm 44.03n$  Da are expected and, indeed, detected. All samples show overlapping distributions with distances of 44.03n Da, the mass of PEG repeat units. All mass spectra can be found in *Appendix A*. It was shown that ammonium prefers larger masses generally in this system, as the distribution favoring ammoniated peaks is 1 to 2 PEG units longer than those of sodiated peaks. This preferential adduction is thought to be due to differing ion sizes. The ammonium ion has an atomic radius of 151 pm [7] while the sodium ion has a radius of 116 pm [12]. It seems appropriate that larger ammonium is less likely to form adducts with shorter chains and lone PEGylated fatty acids due to limited spacing between molecules.

## Summary and Conclusions

Mass spectrometry faces limitations when analyzing complex mixtures due to effects in the ionization and/or detection steps and the inability to distinguish isomeric and often closely isobaric analyte components. LC-ESI-IM-MS was shown to be able to deconvolute a complex nonionic surfactant mixture to characterize it into groups. Overall, the experimentation proved how insightful LC-MS and IM-MS can be to overcome the limitations of MS alone. It was shown that multi-dimensional mass spectrometry is a strong tool by coupling LC with IMS to characterize a polymeric mixture consisting of a glycerol core, PEG, and 2 saturated fatty acids. Multiple groups of components with similar or equal relative polarities would not be distinguishable without separation by size or shape from IMS. This technique is a powerful tool for the chemical process industry, specifically for polymeric product industries (i.e. surfactants, paints, oils, and waxes). Any chemical product that has a mixture of polymers or oligomers with varying conformations can be distinguished relatively quickly with this technique. When optimized for specific systems, LC-IMS-MS can be used in both development applications for verifying structures and in quality control applications to quantify certain characteristics, such as identifying impurities.

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## Appendix A: Mass Spectra

34 ppm CCG in 30% MeOH ACN:Water, Luna Omega C18, with 50 uL of 0.01mg/mL lue-enk, post T in 10 mM NH4OAc  
CCG\_LC\_r1\_C08 219 (3.740) Cm (203.235)

1: TOF MS ES+  
1.17e3

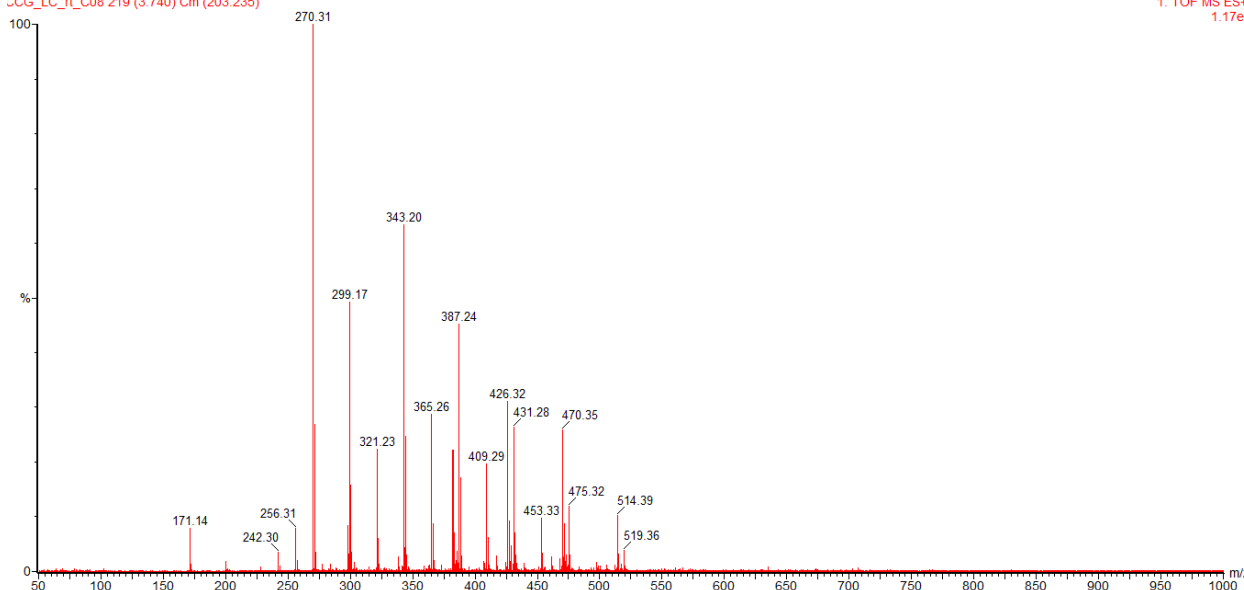


Figure 8 - Mass spectrum of PEGC8

34 ppm CCG in 30% MeOH ACN:Water, Luna Omega C18, with 50 uL of 0.01mg/mL lue-enk, post T in 10 mM NH4OAc  
CCG\_LC\_r1\_C08G1 172 (2.941) Cm (123.225)

1: TOF MS ES+  
2.28e3

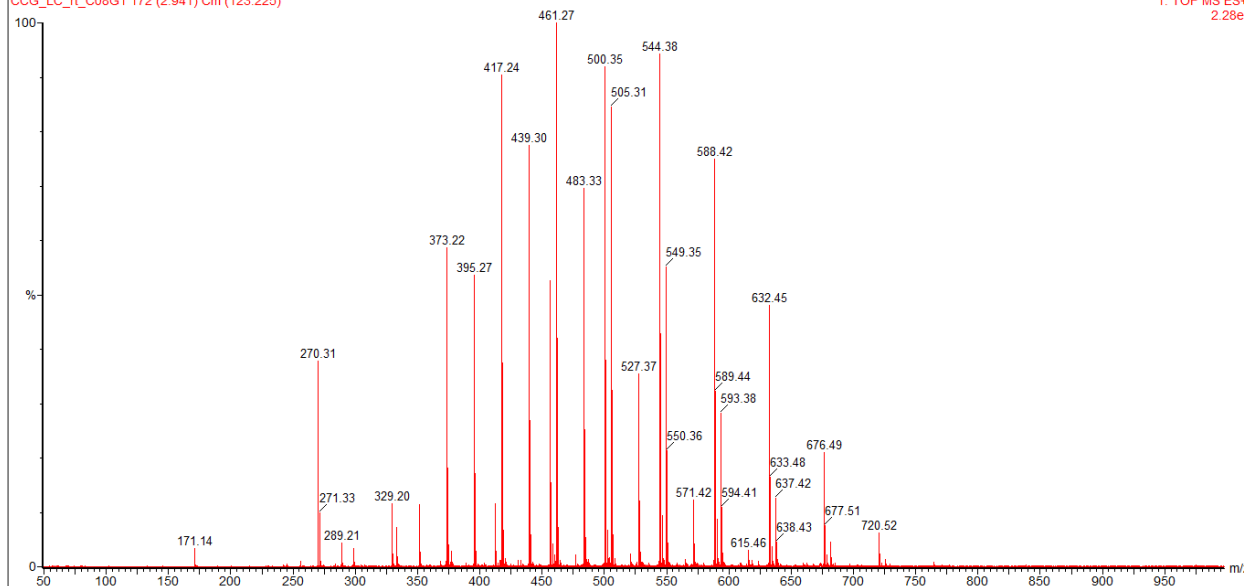


Figure 9 - Mass spectrum of G1PEGC8



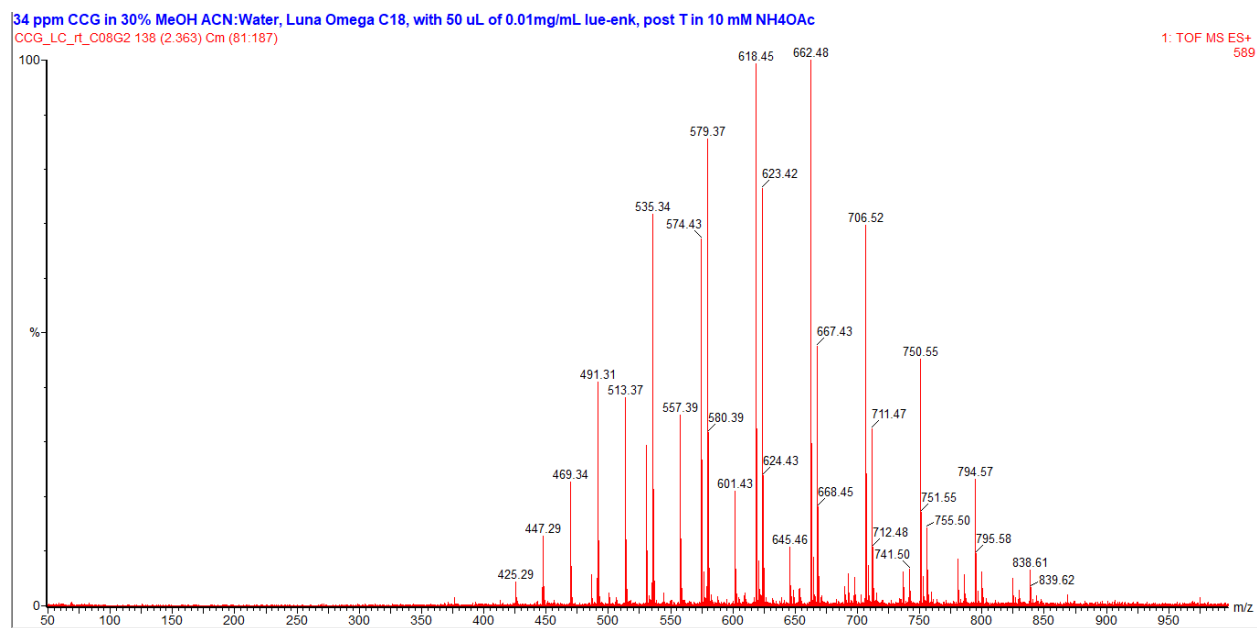


Figure 10 - Mass spectrum of G2PEGC8

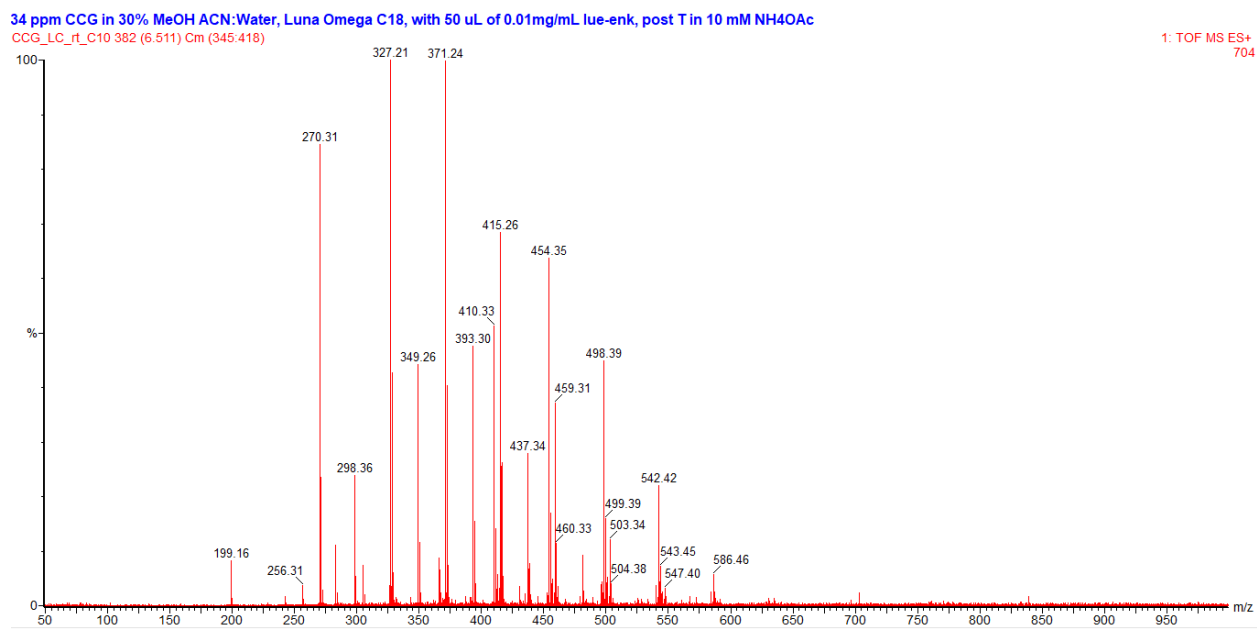


Figure 11 - Mass spectrum of PEGC10

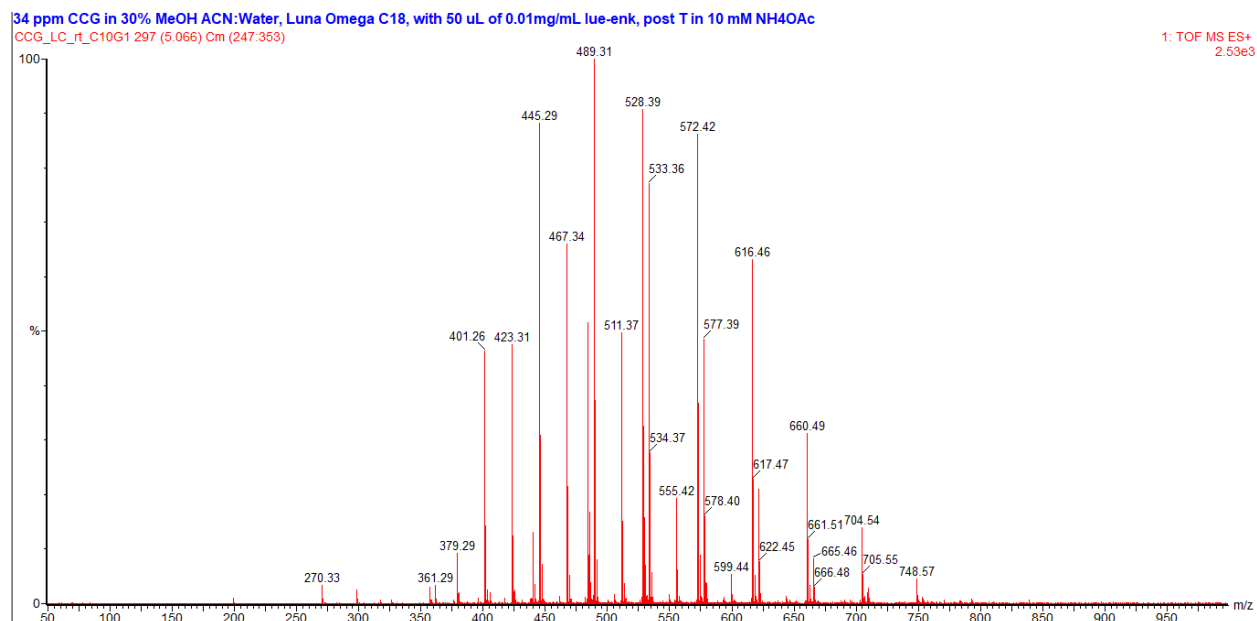


Figure 12 - Mass spectrum of G1PEGC10

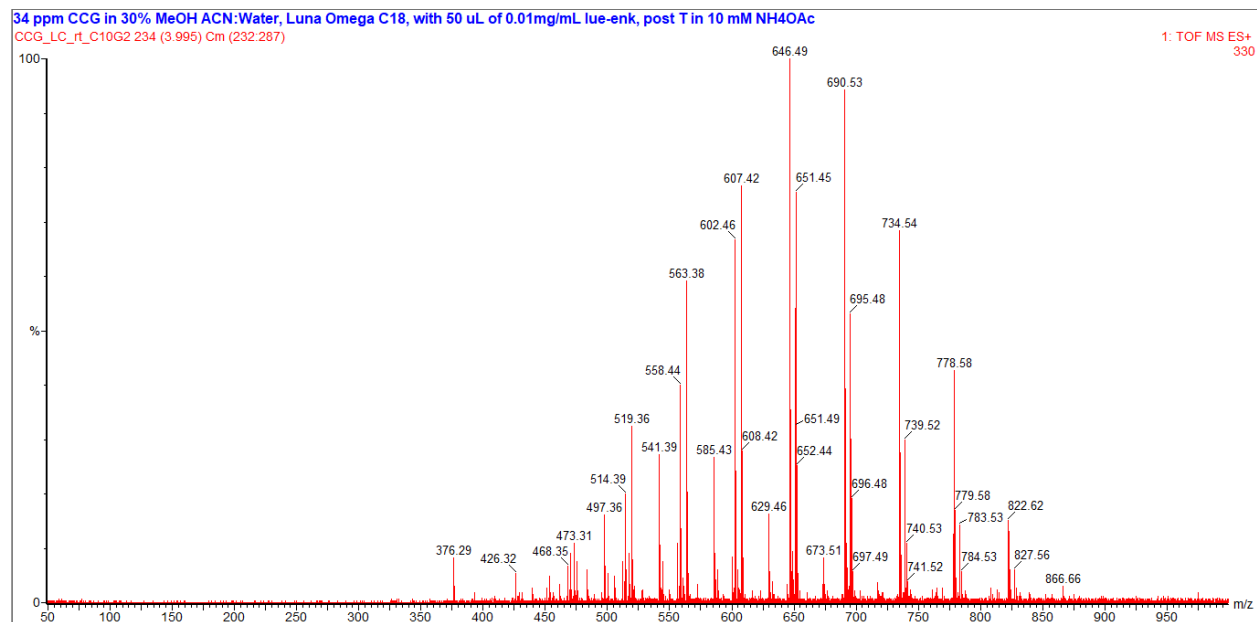


Figure 13 - Mass spectrum of G2PEGC10

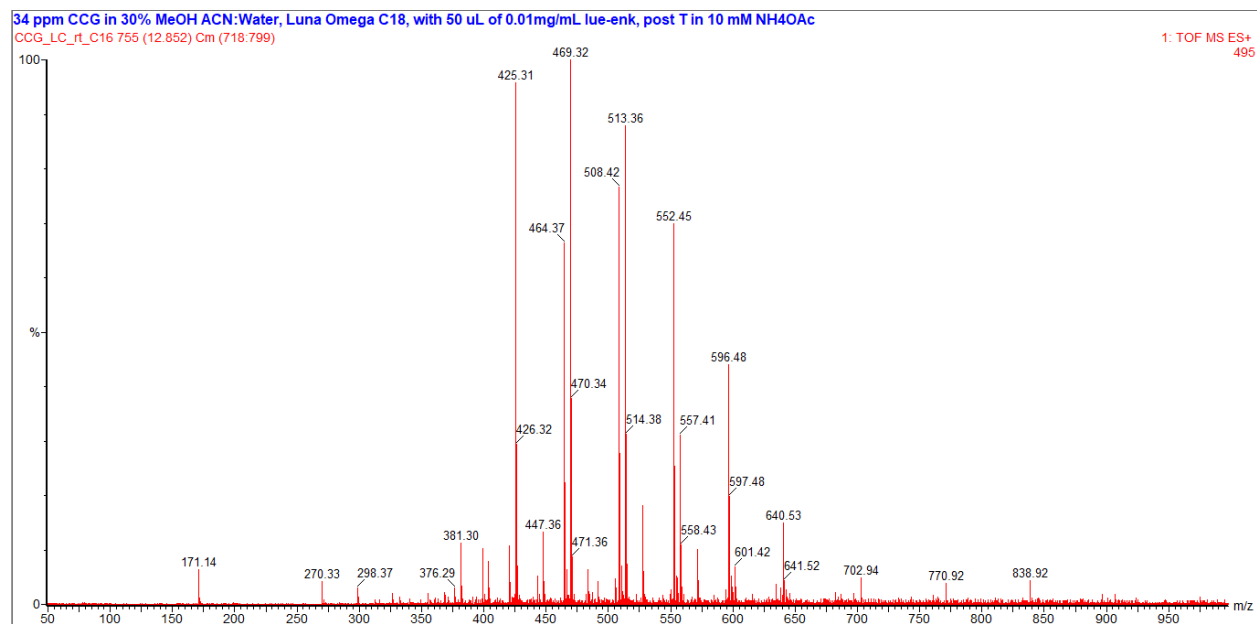


Figure 14 - Mass spectrum of PEGC8C8

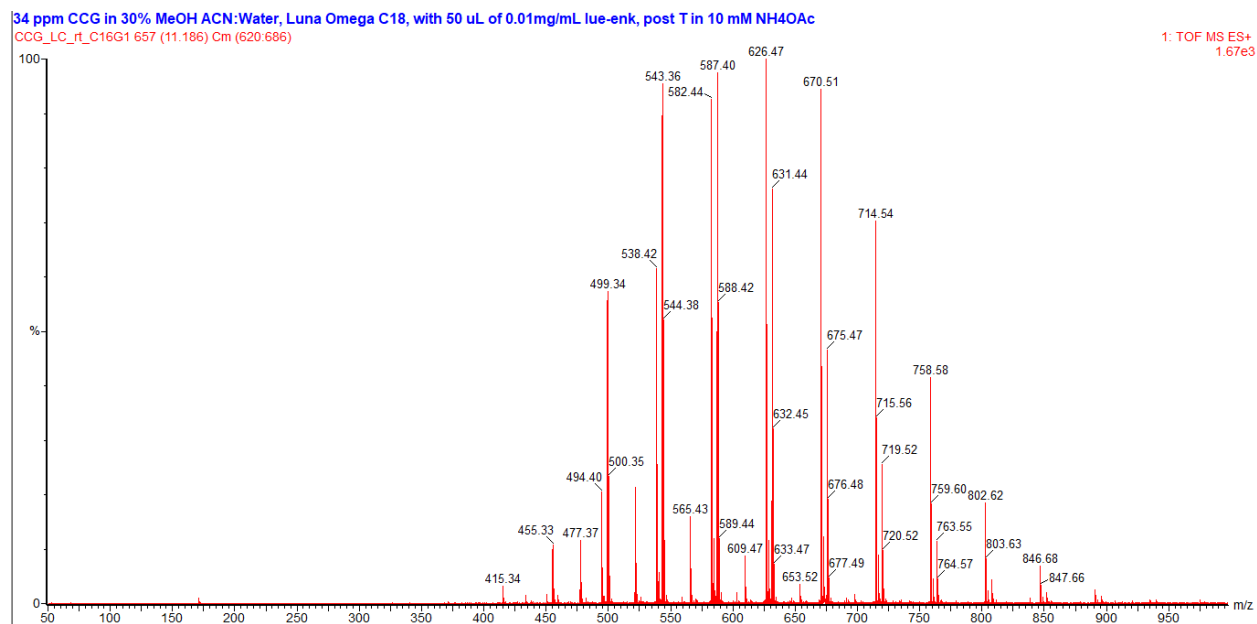


Figure 15 - Mass spectrum of G1PEGC8C8

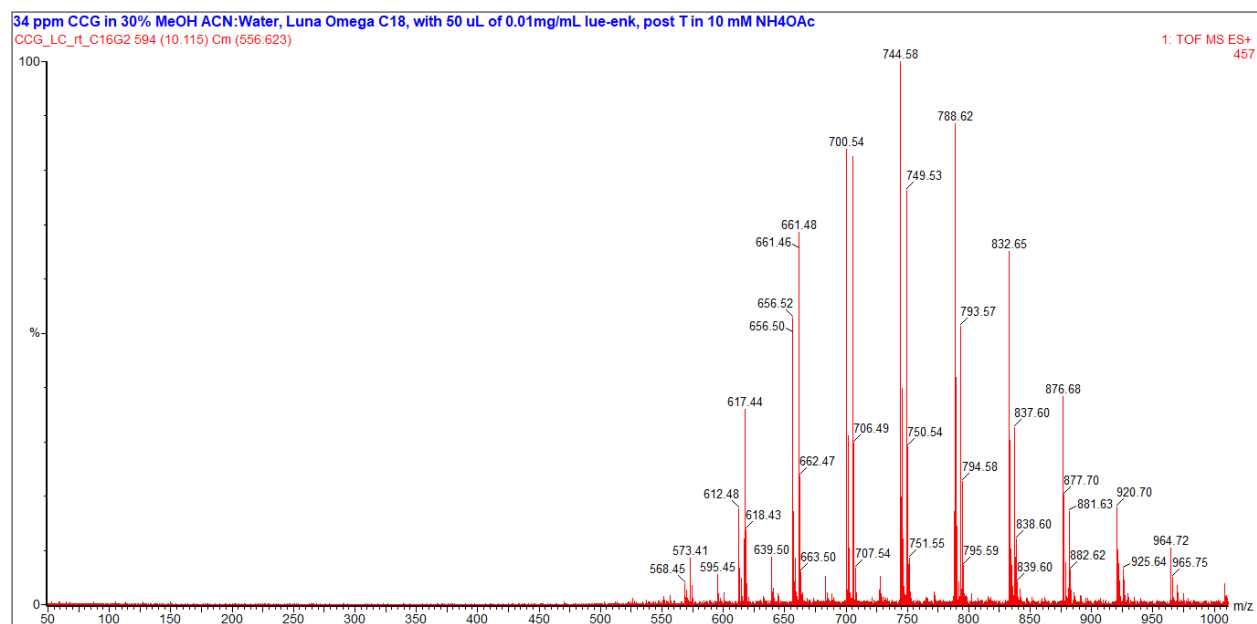


Figure 16 -Mass spectrum of G2PEGC8C8

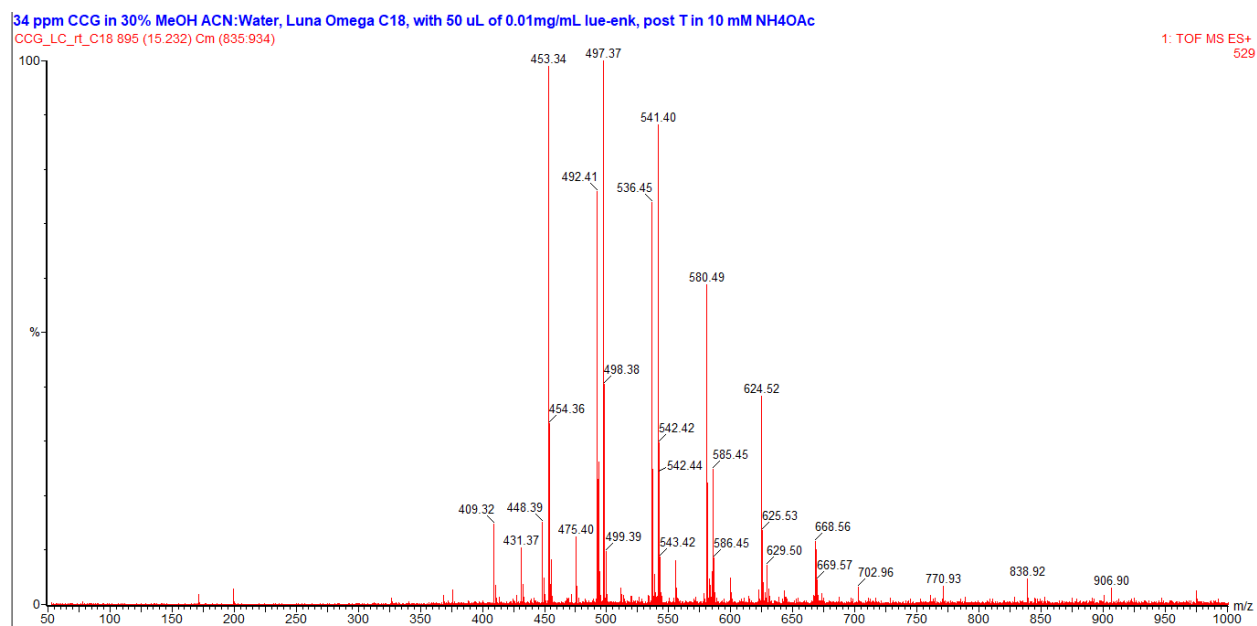


Figure 17 - Mass spectrum of PEGC10C8

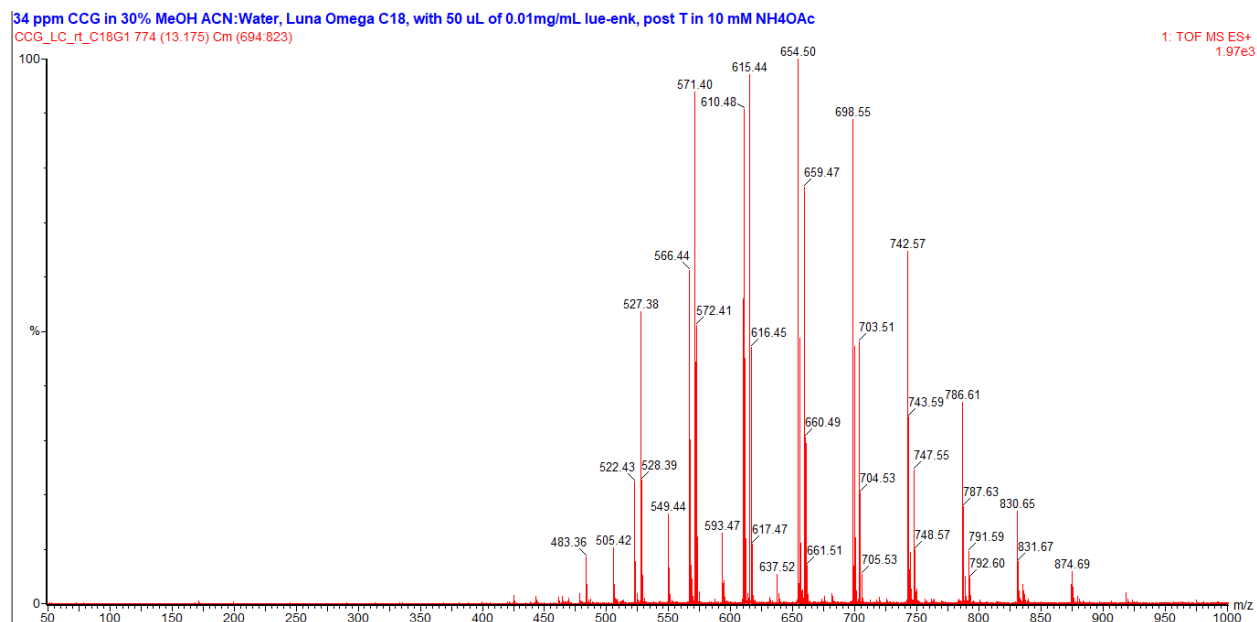


Figure 18 - Mass spectrum of G1PEGC10C8

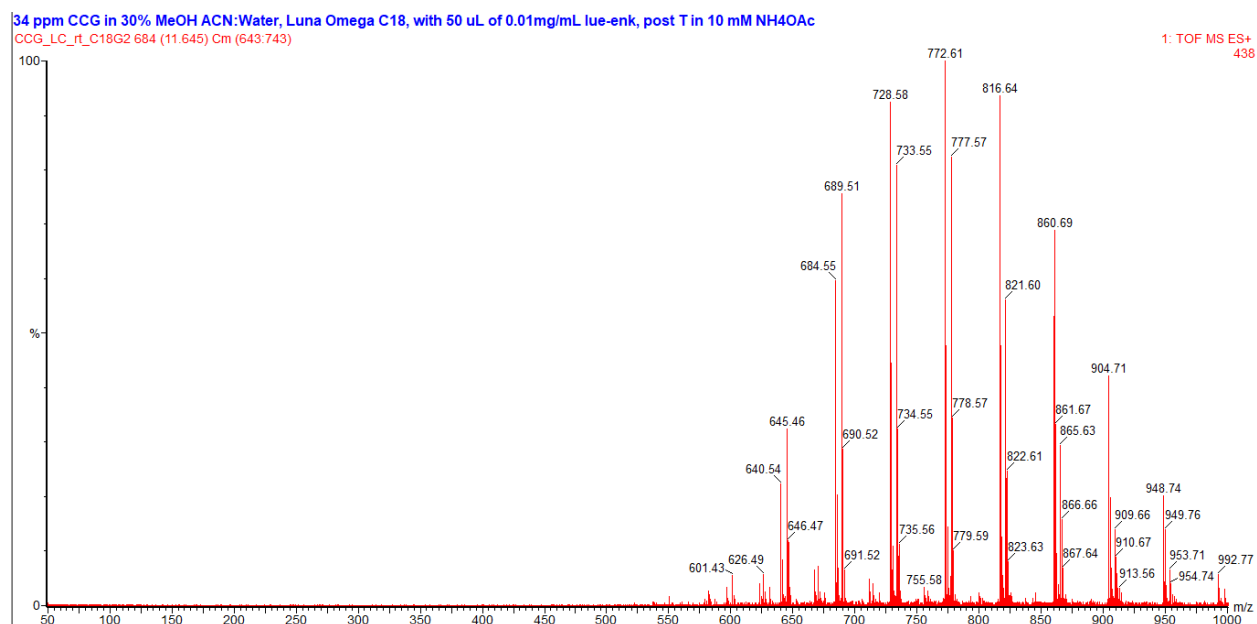


Figure 19 - Mass spectrum of G2PEGC10C8

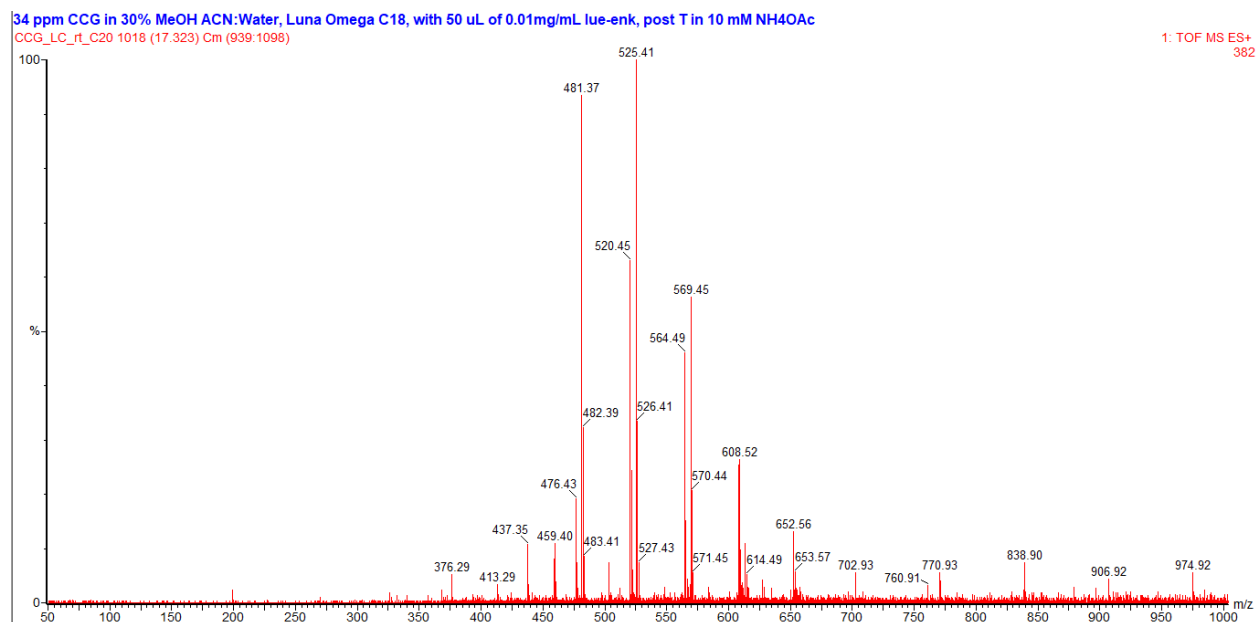


Figure 20 - Mass spectrum of PEGC10C10

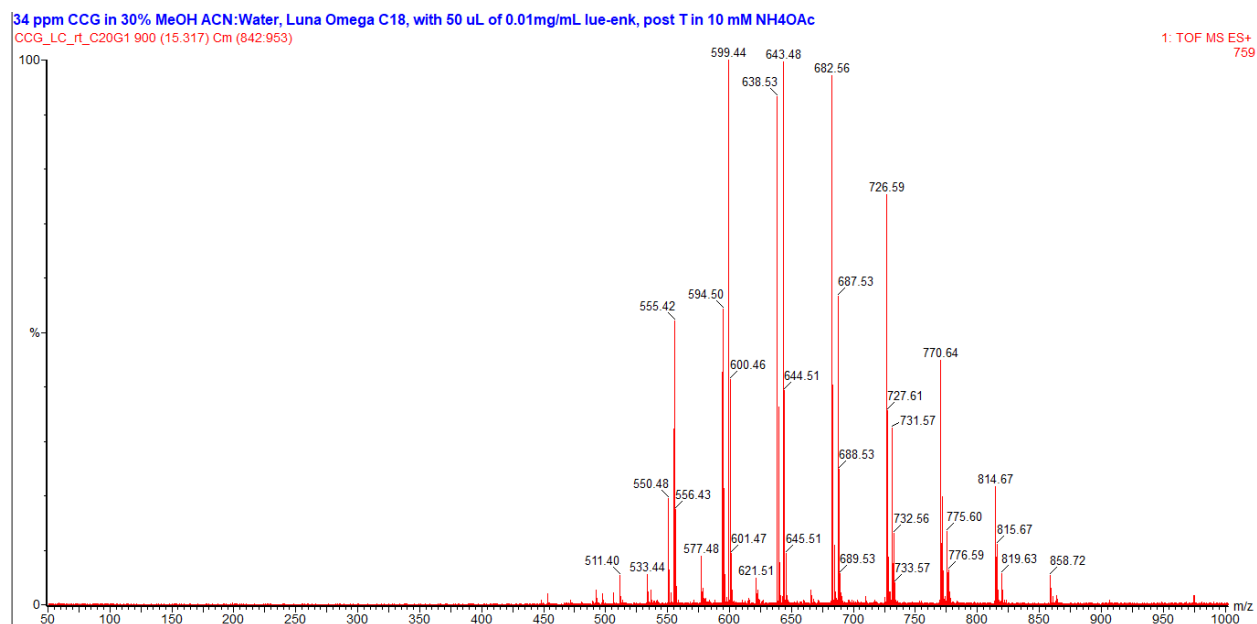


Figure 21 - Mass spectrum of G1PEGC10C10

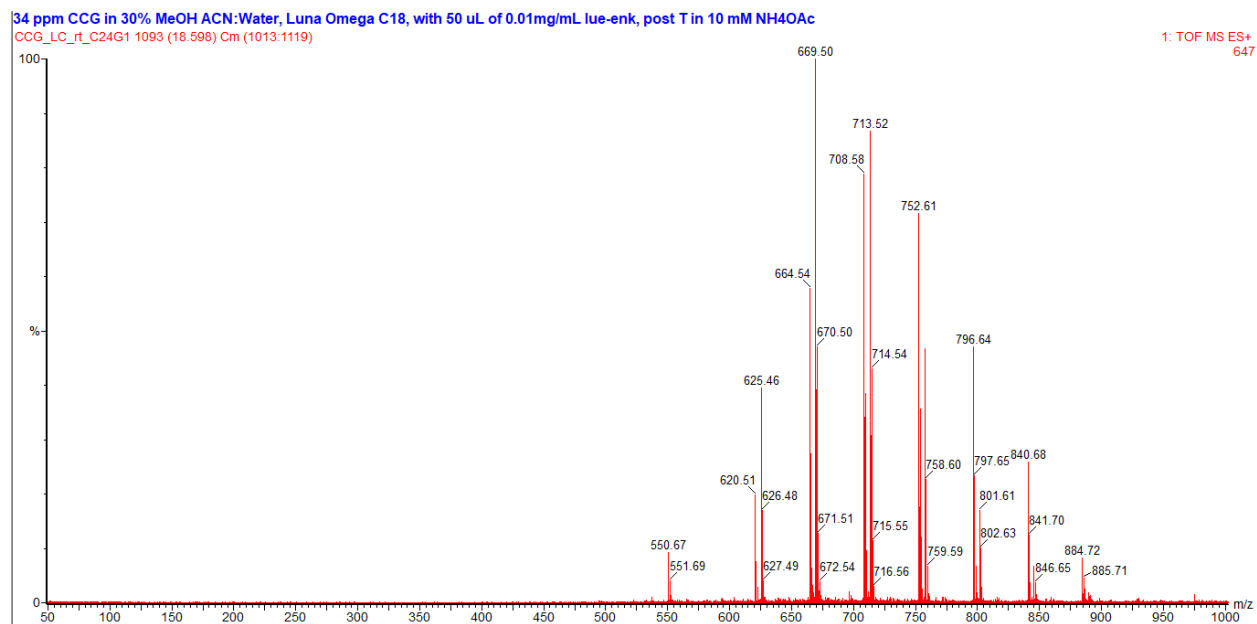


Figure 22- Mass spectrum of G1PEGC8C8C8

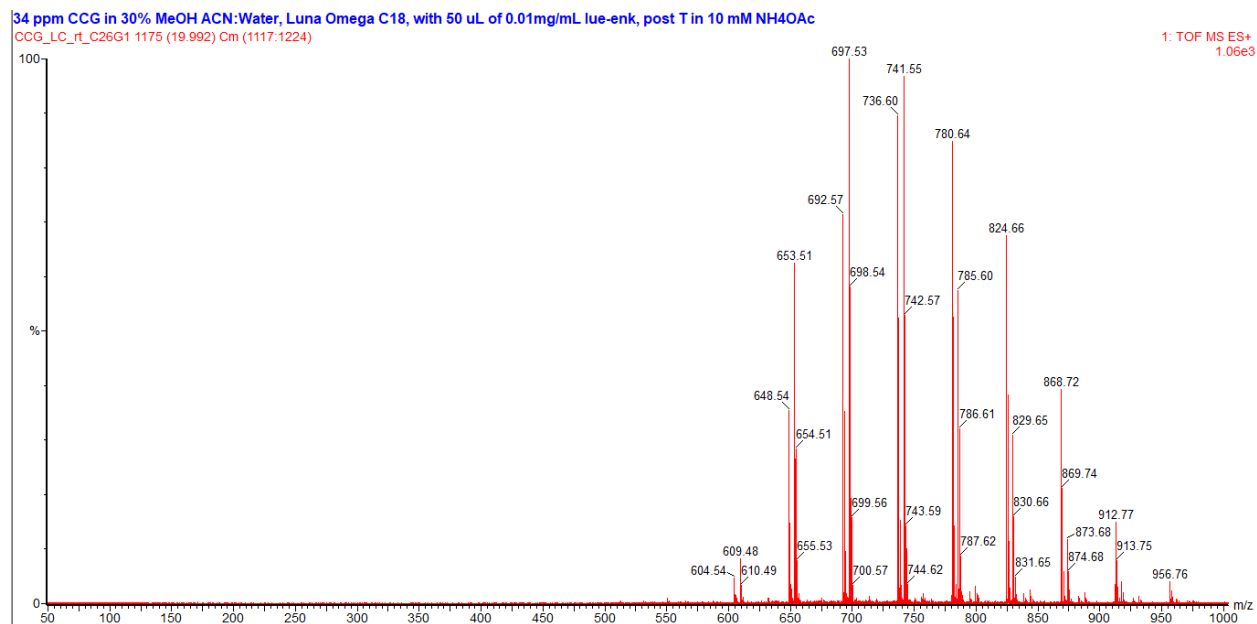


Figure 23 - Mass spectrum of G1PEGC10C8C8

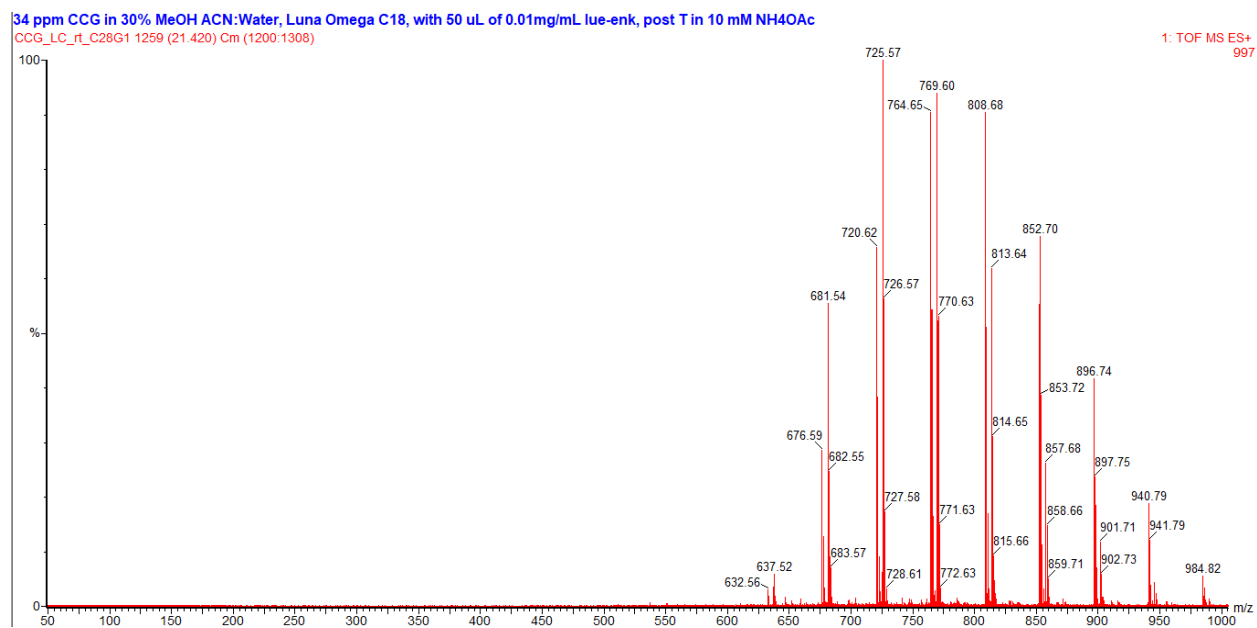


Figure 24 - Mass spectrum of G1PEGC10C10C8

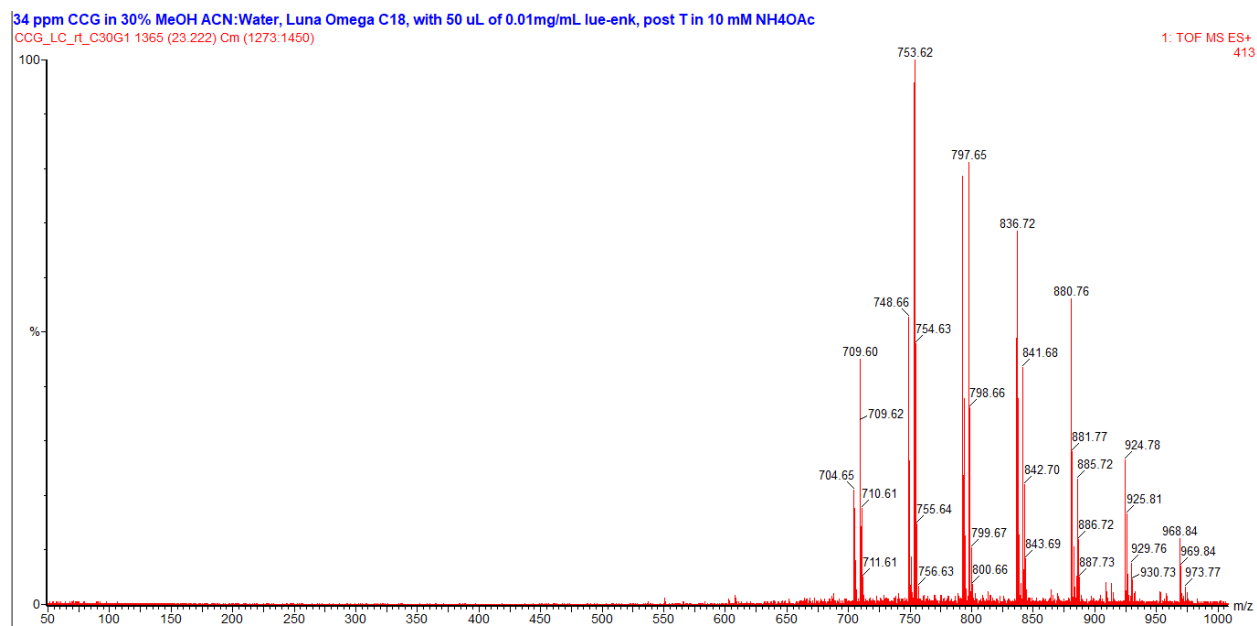


Figure 25 - Mass spectrum of G1PEGC10C10C10