WHY ARE LC VIALS SHOWING GHOST PEAKS WITH THE NEW GENERATION OF MASS SPECTROMETERS?

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CHAPTER 1
LEACHABLES FROM SILICON SEPTUM: INFUSION ANALYSIS

INTRODUCTION

When using an automated GC-MS or LC-MS system, the final extract of a sample preparation protocol is usually transferred into a 96-well plate or a 2-mL glass vial. Those containers are then sealed with a flexible material (silicone septum) to allow easy puncture and reseal ability. With an on-going demand to develop mass spectrometers capable of reaching low sensitivity levels, extraneous peaks will inevitably be detected at levels that may trigger an out-of-trend (OOT) or out-of specification (OSS) investigation[1]. This situation leads to the need for additional analytical work to identify/quantify the cause and ultimately offer corrective measures. In 2000, LC-MS rapidly became the predominant choice for analytical work, thus displacing the decade long tested LC-UV solution. At first, the transition between LC-UV and LC-MS was perceived as seamless primarily because of similar recording traces between a UV analog trace and an MS in digital full scan (MS) or single ion recording (SIR). In short, no extra peaks were being detected above a threshold that would trigger investigative work. However, as early as 2005, reports of ghost peaks[2] with newer generation of mass spectrometers started to show up at levels never seen before. These observations lead to noticeable variations during quantification. In order to keep up with new analytical technology, glass vials manufacturers were encouraged to upgrade their manufacturing workflow and quality control to ensure the final product meets these new demands. As seen in Figure 1, a short 15-minute soak test showed on vial with a significant large mass distribution in the mass-to-charge spectrum, clearly indicating a leaching effect at the final stage of a laborious sample preparation protocol.

The term "extractables" and "leachables", or E&L, refers to compounds that can be extracted under extreme conditions (harsh solvent, high temperature, etc.) and to compounds that can migrate or leach by direct contact under normal conditions[3]. Three major business segments are directly affected by the omnipresence of E&L: Food Safety, Pharmaceuticals, and Packaging Manufacturers. The presence of leachables in the food industry came to public light when the Canadian Ministry of Health banned polycarbonate infant bottles, fearing potential exposure to bisphenol A (BPA). In the pharmaceutical industry, containers are not the only source of leachables; drug products such as formulations, fillers, and suspensions are also potential sources. Since 1999, the US Food and Drug Administration (FDA) provided guidance for protection against extractables and leachables with its "Guidance for Industry: Container Closures Systems for Packaging Human Drugs and Biologics".[4,5] In 2005, the European Agency for the Evaluation of Medical Products (EMEA) issued the "Guideline on Plastic Immediate Packaging Materials".[6]

From a workflow point of view, E&L analytical protocols utilize a wide range of extraction, separation, and detection techniques to meet FDA or EMEA regulations. As seen in Figure 2, a controlled extraction study will select options from various solvents to cover a wide polarity range and use several solid-liquid extraction techniques to produce several extracts for analysis by GC-MS (volatile) and LC-MS (non-volatile)[3,7,8,9,10,11]. During the extraction process, contact time, additives, and temperature are key parameters to ensure maximum exposure with the extraction solvent.

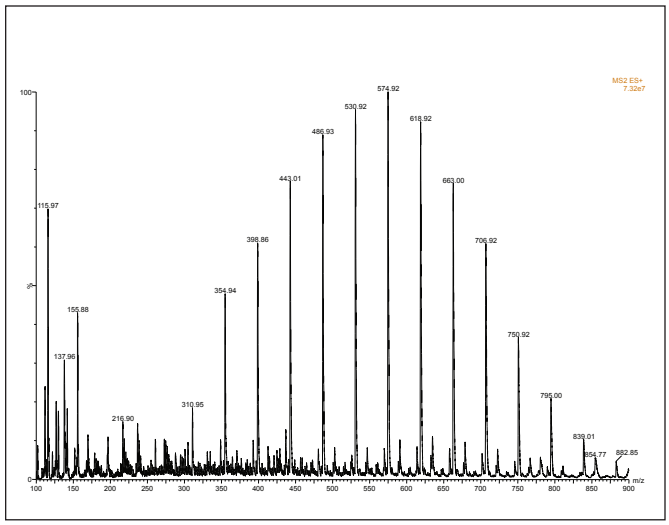

Figure 1. Leachable ion distribution from a silicon cap soaked in methanol.

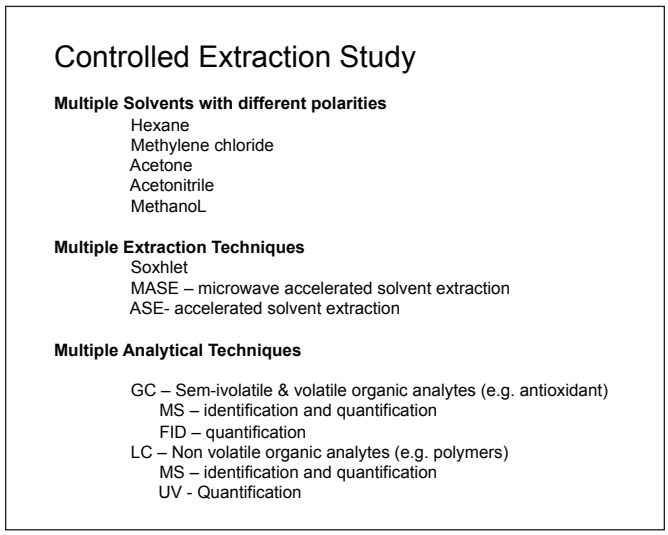

Figure 2. Current extraction protocol and techniques for leachable experiments.

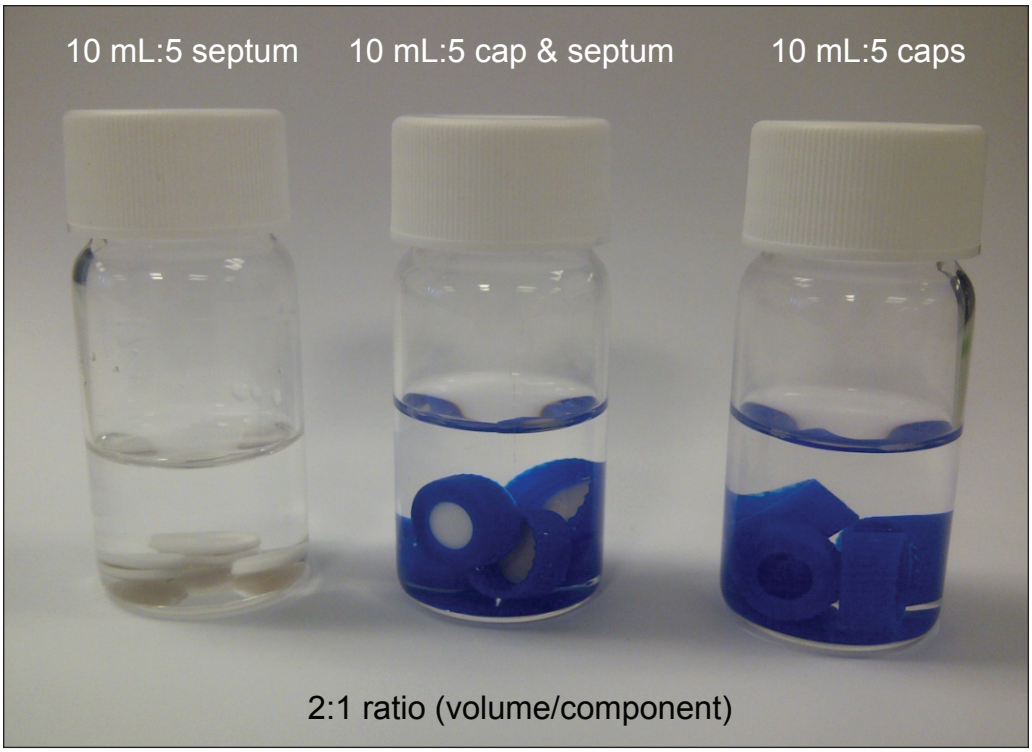

Figure 3. Soak experiment for silicon septum and caps.

EXTRACTION PROTOCOLS

With regard to solid sample extraction techniques, several choices are available, from the century-old Soxhlet extractor, to heated reflux, as well as the time-saving accelerated solvent extraction (ASE) using elevated temperature and pressure (similar to Soxhlet principle). Recently, a relatively new technique utilizing electromagnetic waves as a heat source has been introduced – the microwave accelerated solvent extraction (MASE), which uses closed-extraction vessels and claims fastest extraction times. All techniques are designed to accommodate sample size from small (1 g) to large scale (1000 g). The choice of solvent for extraction will dictate which analytical technique will be used for final analysis.

Currently, liquid and gas chromatography coupled with mass spectrometry are the top analytical choices for analysis. Since extraction techniques can use various solvents to cover a wide polarity range, polar extracts are directed toward LC-MS analysis, and non-polar extracts are typically analyzed by GC-MS. With single-dimension chromatography (LC or GC), the final solvent composition is crucial to ensure proper sample focusing during the injection process. This requirement will produce Gaussian peak shape ideal for qualification/quantitation analysis. However, to achieve acceptable quantification performance at trace level

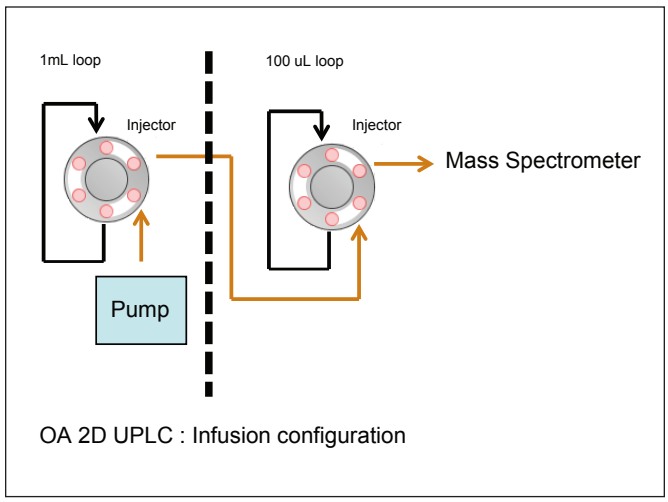

Figure 4. Open Architecture 2D-LC in infusion mode.

(sub ppb), the extraction protocol usually includes a large sample enrichment process. Since current LC-MS and GC-MS are still limited to small injection volumes for analysis, extraction protocol must include a sample volume reduction and a solvent conversion step at the end of the extraction process.

The evaporation and reconstitution step is usually achieved with rotor-evaporators with reduced pressure or by using the gentle nitrogen gas stream technique. In each case, evaporative loss

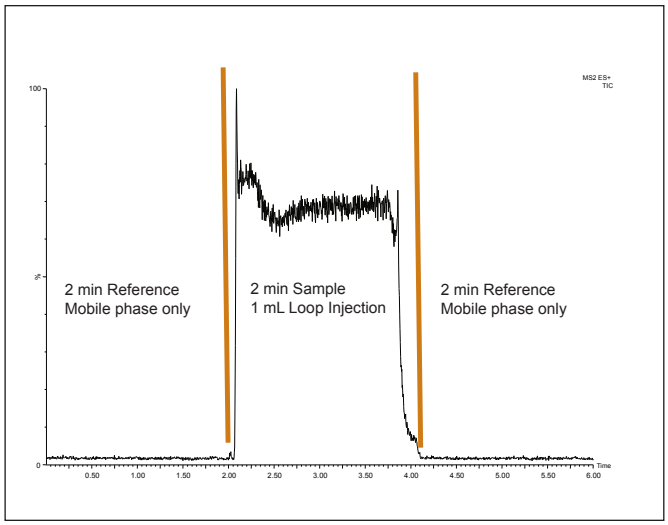

Figure 5. Total ion chromatogram with pre- and post- reference sections.

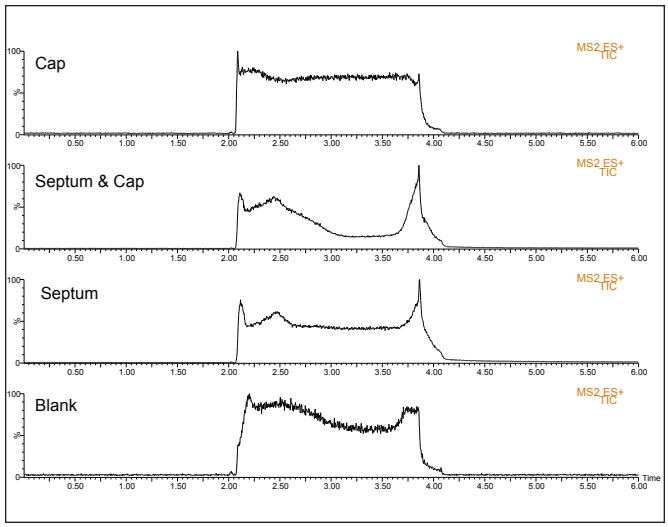

Figure 6. Infusion TICs for septum, cap, and septum/cap profile.

and redissolving issues can occur and cast various levels of uncertainties on the final results. Regardless of the extraction technique, the evaporation/reconstitution step is usually regarded as time-consuming and extremely laborious. With the initial sample volume and final extract volume, an enrichment ratio can be factored during quantification. Since small injection volumes (ex: ≥10 µL LC/MS and ≥1 µL GC/MS) are still used for analysis, macro-extraction protocols are quite inefficient. In fact, it means that only 1 % of the final extract (typically 1 mL) is used for measurement and, therefore, 99% of the total work used during the extraction process is simply discarded.

EXPERIMENTAL

As stated earlier, leachables refers to entities that can migrate by direct contact under intended conditions. The solvent volume-to-mass ratio is crucial in order to ensure complete sample coverage. A typical leachable experiment can utilize variable mass-to-volume ratio (i.e., 1:1, 1:10, 1:100 or 1:1000). With the larger ratio, the contact solvent will inevitably needed to be evaporated to dryness and reconstituted in an appropriate solvent for further analysis (LC-MS or GC-MS). For potential leachables present in re-sealable silicone cap used with 2-mL glass vial, septums were placed in a clean 20-mL container with four solvents (water, methanol, acetonitrile, and acetone) for a 60-min time period. The volume-to-mass ratio chosen for this experiment was 2:1, which translate to 5 caps in 10 mL total solvent. The containers were sealed and let at room temperature before analysis (see Figure 3). The 20-mL vials with caps were transferred into a large plate holder and no additional handling was performed with the sample.

The infusion analysis was performed using an Open Architecture UPLC® System with 2D-LC Technology set in "infusion mode." As seen in Figure 4, a 1-mL total sample were aspirated from the 20-mL vial and injected into a 1-mL loop. The infusion analysis collects a 2-min reference signal from the loading pump using a 50:50 water:leach solvent with 0.5% formic acid. After the 2-min reference, the injection loop was pulsed into the infusion stream for a full scan acquisition (100 to 1000 amu) under positive electrospray. With a loading flow rate of 0.5 mL/min, the content of the injection loop was flushed completely after 2 min. At the four-minute marker, the injection loop was pulsed into injection mode and the infusion analysis continued with another two minutes reference. As shown in Figure 5, a total ion chromatogram (TIC) shows the pre and post reference section, with target sample in the middle. At this point, 500 spectrums were combined for analysis.

RESULTS

Infusion analysis

The objective of this research was to evaluate the leachables content of pre-slit PTFE/silicone seal cap for 2-mL glass vials. This study was triggered by an increase in reports from users observing sudden appearance of ghost peaks during method

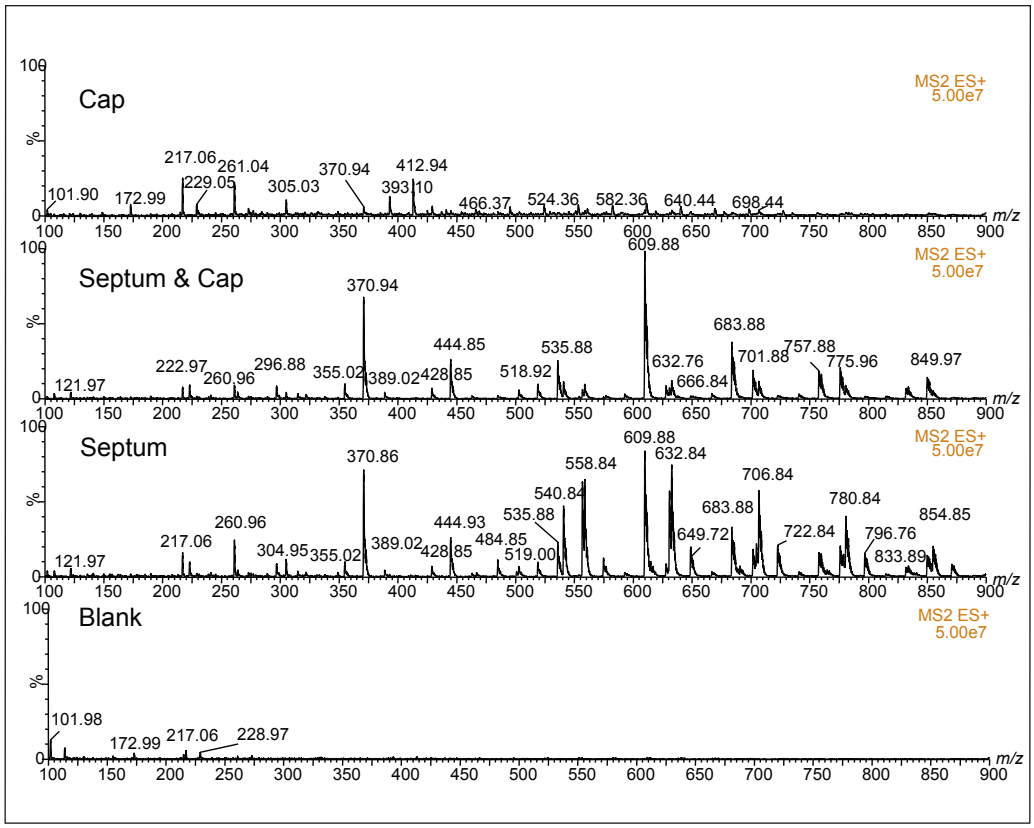

Figure 7. Combined spectrums for septum, cap, and septum/cap.

development and routine analysis. From this point, two multi-dimension chromatography configurations were used to identify leachables present in resealable silicone disks (infusion mode) and to gain insight on chromatography behavior with two-dimensional chromatography with At-Column Dilution mode (2D with ACD mode).

In the infusion mode, a selection of 2-mL caps was picked for leachables study. The re-sealable 2-mL caps are made of a silicone pre-slit material with a Teflon backing acting as an inert barrier. Those silicone caps are currently the preferred format primarily for the option of repeated injections without the need to replace the caps and reduction of evaporative lost effect. Other materials were included in the selection, such as the single injection PE or PTFE caps, and several substitute materials offering flexibility and potential re-sealability. The investigative work started by measuring which part of the cap assembly, the plastic cap or the silicone septum, is prone to leaching. Figures 6 and 7 are showing the TIC and the combined spectrum for: a methanol blank; septum only; septum and cap; and finally, the cap only. The spectrums clearly indicate that the silicone septum is prone to leachable effect, while the plastic cap shows no signal.

The next objective was to determine which solvent has the highest solubility for leachables. In this instance, since these caps are targeted for reversed-phase chromatography applications, water and water-soluble solvents (methanol, acetonitrile, and acetone) were chosen for their compatibility in both sample preparation protocols and chromatography conditions. As seen in Figure 8, the TIC for water shows a weak signal. However, the signals are very strong for methanol (MeOH), acetonitrile (ACN), and acetone (ACE). The combined spectrums show two distinct signal types: a multiple-charge distribution signal with repetitive ions (i.e., 610, 684, 758, 832, etc.); and the single-charge species (i.e., 371). The multiple-charge distribution is a tell-tale signal of polymer entities, which can be de-convoluted for total mass calculation. The ion distribution and intensity between MeOH, ACN, and ACE indicates that leachables components of the silicone septum are highly soluble in a polar solvent. This result suggests that the leachables seen in the MeOH spectrum could have a polar characteristic and potential elute in the early portion of gradient elution with reversed-phase chromatography, thus causing potential matrix effect.

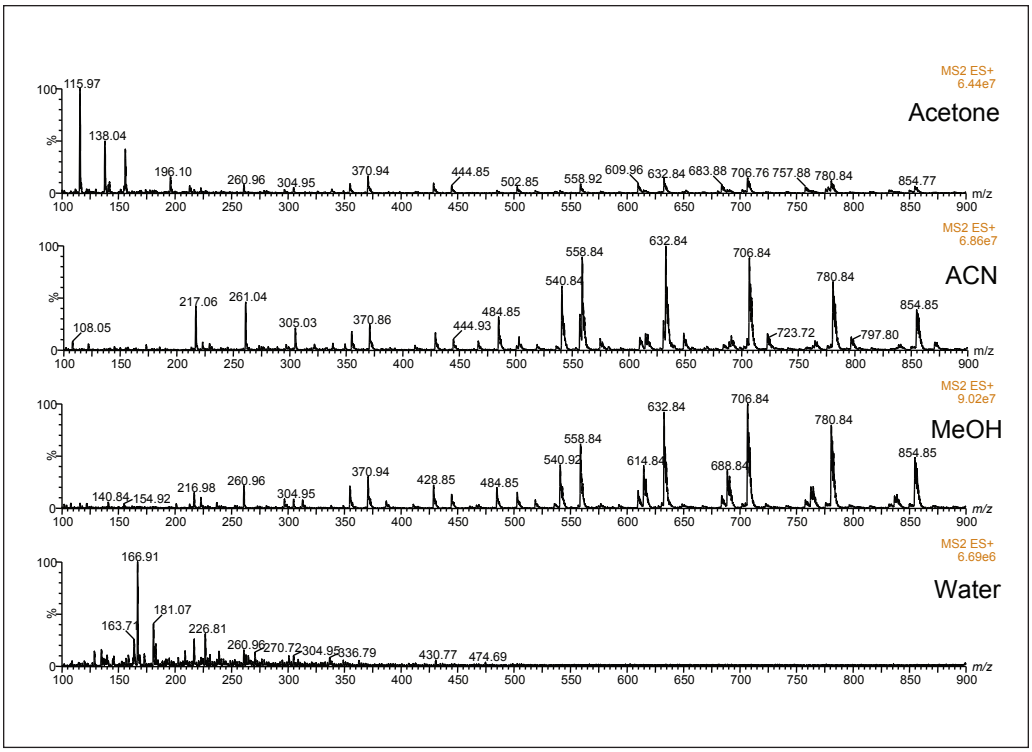

Figure 8. Combined spectrums for water, methanol, acetonitrile, and acetone for silicone septum extract.

The combined spectrums in Figure 9a, 9b, and 9c show the MeOH leachable extracts for 12 2-mL caps; showing results for two single-use only plastic septum, four silicone septum, three competitor silicone septum, and three alternative materials. The results clearly indicate that the PE and PTFE septum show the lowest leachables levels. This result was expected from this type of material under these mild analytical conditions. The single-use cap offers the best performance with respect to leachable levels. Since most applications require replicate injections for reproducibility data, this option has limited applicability. The PTFE/silicon septum remains the industry norm. Proceeding with the analysis, of the seven silicone septum tested, all tested positive for the same 610 multiple-charge series with one exception, which tested negative. This new formulation is the result of an optimized manufacturing procedure in response to high-sensitivity mass spectrometers. Of the three alternate materials, none gave satisfactory results.

CONCLUSIONS

In this application, leachable experiments were conducted with minimum manual labor. The ACQUITY UPLC® System with 2D-LC Technology[12,13] with infusion and at-column dilution configurations enabled 500:1 enrichment analysis by using large-volume injections (aqueous and organic). These two configurations eliminated the time-consuming evaporation-to-dryness and reconstitution steps.

From the results of the experiment, we see there are different levels of extractable masses from different polymers. Some of the polymer materials are not acceptable for MS, as they leach too many masses using solvents common to reversed-phase chromatography.

Within the same septa material (PTFE/silicone) from different suppliers, the levels of leachables using the same solvent conditions yield different results. All manufacturers and suppliers have not achieved the same levels of process and quality controls to offer product clean enough for sensitive MS instruments.

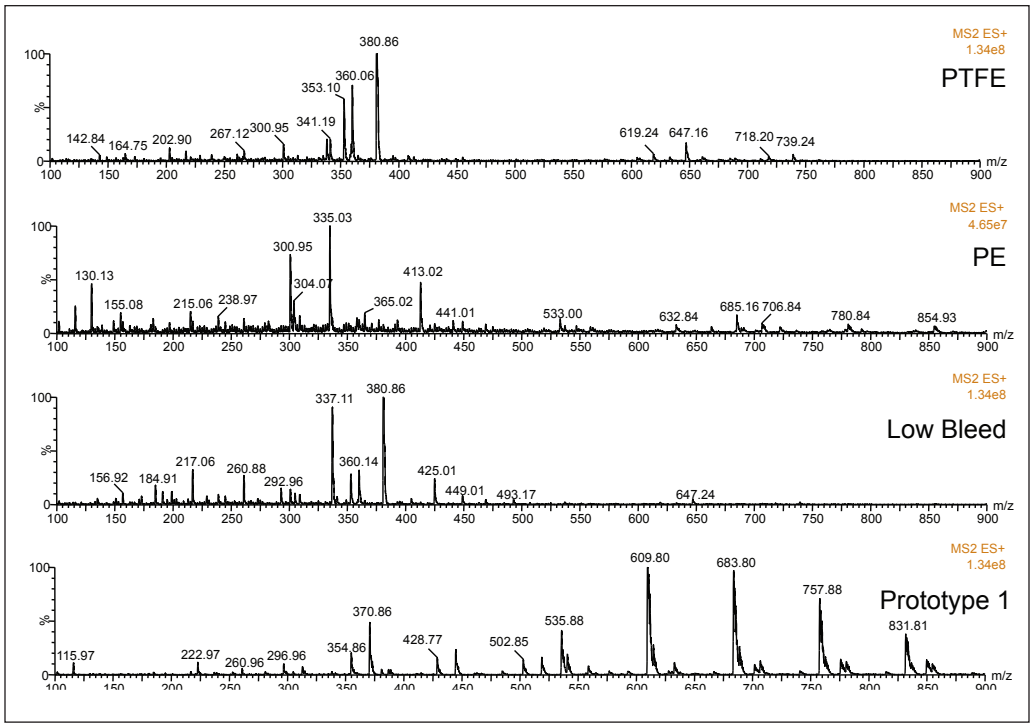

Figure 9a. Combined spectrums (methanol extract) for PTFE, PE, low bleed, and prototype I septums.

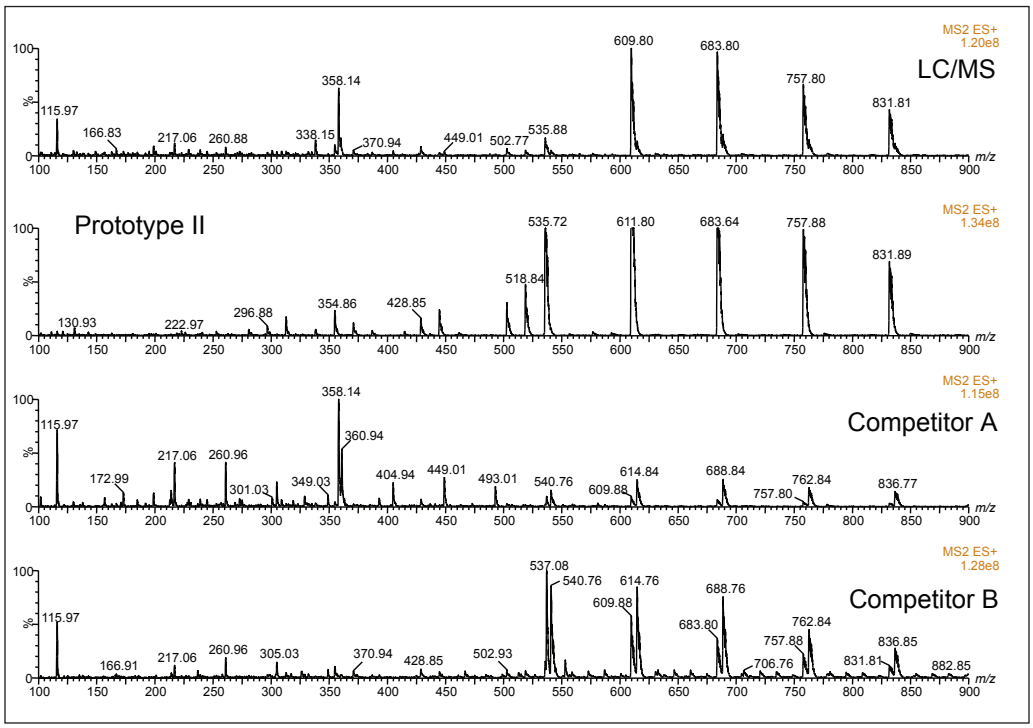

Figure 9b. Combined spectrums (methanol extract) for LC/-MS certified, prototype II, competitor A, and competitor B septums.

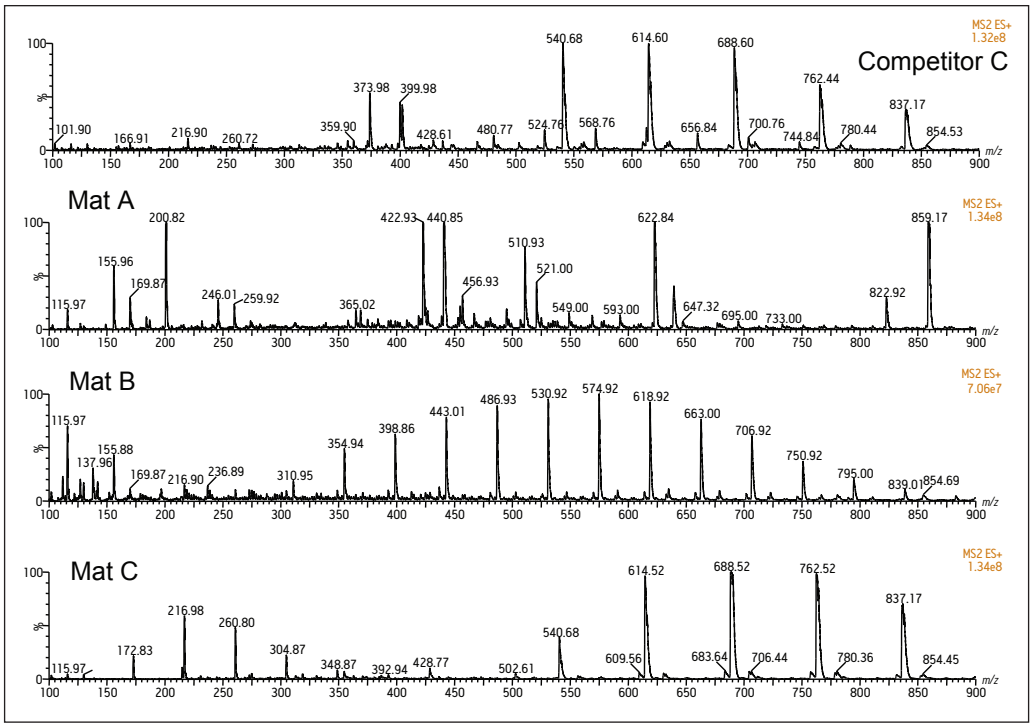

Figure 9c. Combined spectrums (methanol extract) for competitor C, material A, material B, and material C.

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