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

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CHAPTER 3
LEACHABLES FROM VIALS: CHROMATOGRAPHY

INTRODUCTION

This is the third installation of leachables experiment using Open Architecture UPLC System with 2D-LC Technology and at-column dilution.[1,2] In this experiment, we further explore the technique by soaking different brands of vials in different soak solvents, then run all extracts in acetonitrile gradients and discuss results. This experiment reinforces the ability to process many samples and solvents in a short timeframe.

EXPERIMENTAL

For the enrichment analysis, the Open Architecture UPLC System with 2D-LC Technology was upgraded with the at-column dilution option1. The chemistries used for D1 and D2 were the Oasis HBL 20 µm (2.1 x 30 mm) and the BEH C18 1.7 µm (2.1 x 50 mm) columns, respectively. The loading conditions used for at-column were set at 5% dilution (loader pump at 0.2 mL/min and dilutor pump at 4 mL/min). The injection volume was set at 500 µL for a 4-min loading time. The trapped analytes were back flush eluted with a 0.5 mL/min gradient. The elution started at 5% to 95% organic for 5 minutes with 0.5 % formic acid. Three organic modifiers were used for the chromatography (methanol, acetonitrile, and acetone). The mass spectrometer was set under scan mode (100 to 1000 amu) with positive electrospray (ESI). Each 2-mL silicone cap extracts (water, methanol, acetonitrile, and acetone) were subjected to all three chromatography conditions. The 2-mL vials leachable experiments were conducted with the same protocol with one exception: the vials were covered with an aluminum foil to remove the potential contribution of the septum cap.

RESULTS

After a 30-min contact period, five 2-mL vial replicates, from three different vial brands, were individually analyzed for the methanol, acetonitrile, and acetone leaching experiments. The results are presented in Figures 1a, 1b, 1c, 1d, 1e, and 1f, respectively. The chromatograms on the left side are total ion current chromatograms (TIC's) with a 5-min acetonitrile gradient from 5% to 95%, starting at 4 minutes. From time zero to three minutes, each extracts were loaded onto the trap dimension for enrichment. The spectrums on the right are combined spectrums, indicated by the red arrows. The baseline profile is typical of an acetonitrile gradient, with increasing values of the organic modifier. The distinction from one vial to another can be seen from additional well-resolved peaks and also mild to severe baseline distortion. In this case, vial 1 shows a high level of baseline distortion and well-resolved peaks across all three leaching experiments. The methanol leaching shows a higher number of gaussian peaks, while the acetone leaching shows the severe baseline distortion at 6.82 minutes. With vial 2, the methanol leaching shows a milder baseline distortion with no extra peaks, while the acetone leaching shows a comparable profile with vial 1. Vial 3 shows minimum contribution across all

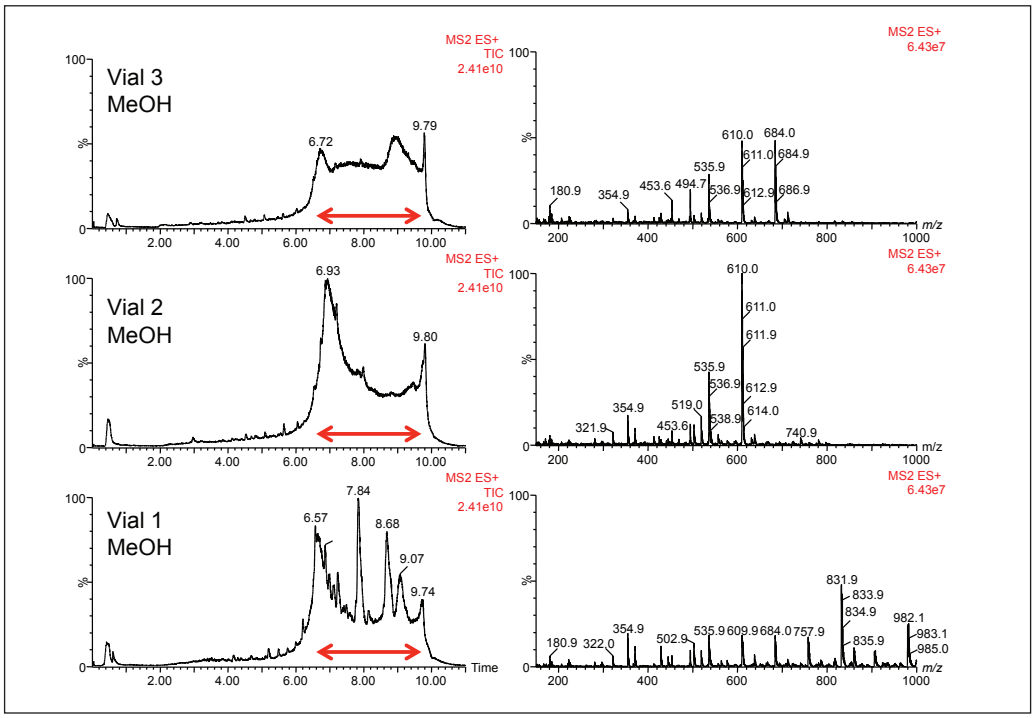

Figure 1a. The chromatograms are total ion current chromatograms (TIC's) in an acetonitrile gradient. Three, 2-mL vial brands soaked in methanol for 30 minutes (5 replicates for each vial brand), covered with aluminum foil and the extract was run on an Open Architecture UPLC System with 2D-LC Technology and at-column dilution. The spectra on the right are the combined spectrum within the red arrows of the chromatogram on the left. The rising baseline is typical for acetonitrile at increasing organic level. Vial 1 shows distinguished Gaussian peaks after 8.5 minutes. At this time, the gradient is high organic showing high reversed-phase retention. Vials 2 and 3 did not show the peaks.

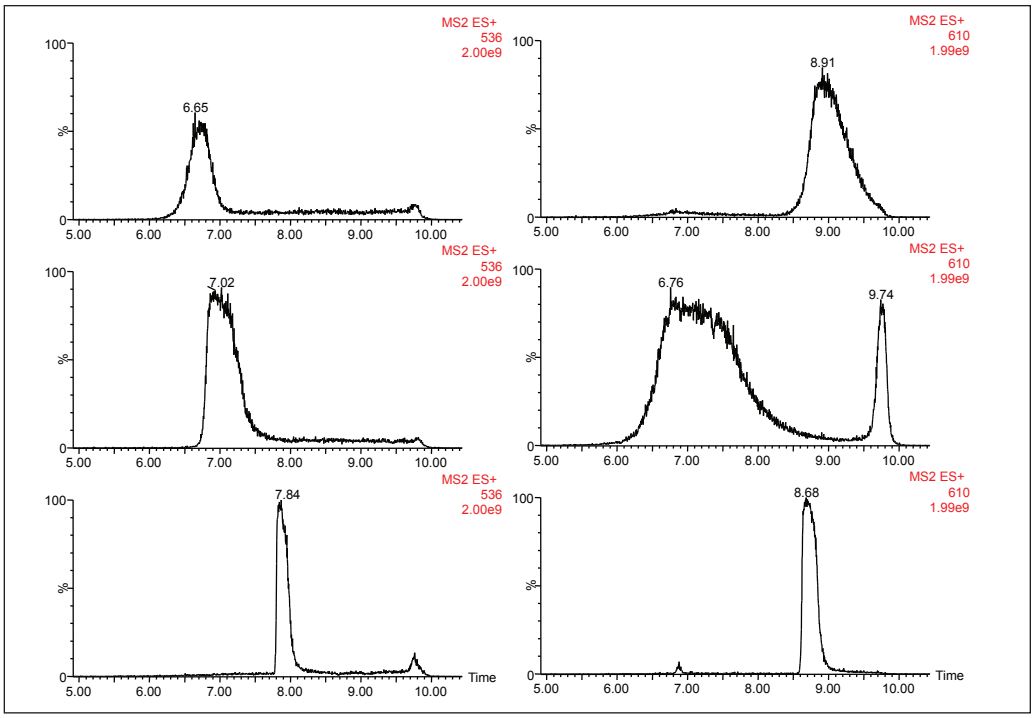

Figure 1b. Two ions were extracted from the TIC, 536 and 610 from figure 1A. 536 ion was well resolved at 7.84 minutes in vial 1; vials 2 and3 have different retention times and wider chromatography profiles. This suggests that the 536 ion, although the same parent mass, could be three different entities.

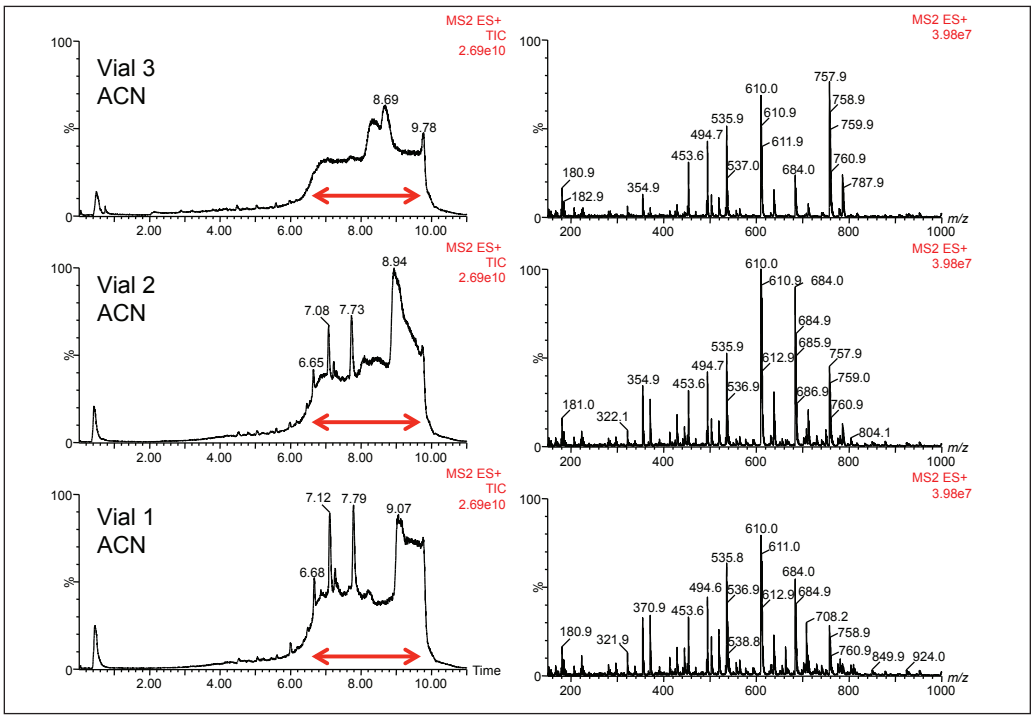

Figure 1c. Three, 2-mL vial brands soaked in acetonitrile for 30 minutes (5 replicates for each vial brand), covered with aluminum foil and the extract was run on an Open Architecture UPLC System with 2D-LC Technology and at-column dilution. The chromatograms on the left were run in an acetonitrile gradient. The spectra on the right are the combined spectrum within the red arrows of the chromatogram on the left.

three leaching experiments, which suggests low leachable entities on the glass surface. From the combined spectrums, extracted mass chromatograms can help visualize the chromatography profile of most abundant ions (m/z). For the three vials tested in this experiment, the 536 and 610 ions were extracted from the TIC and presented in Figure 1b (methanol), Figure 1d (acetonitrile), and Figure 1f (acetone). From this data set, the chromatographic behavior of the selected ions can be traced and compared between vial 1, 2, and 3. The 536 ion is seen as a well-resolved peak at 7.84 in the vial 1 methanol extract. However, the same ion is at different retention times and wider chromatography profiles in vial 2 and 3. This could suggest that the 536 ion, although the same parent mass, could be three separate entities. In this case, further MS/MS characterization can add additional information

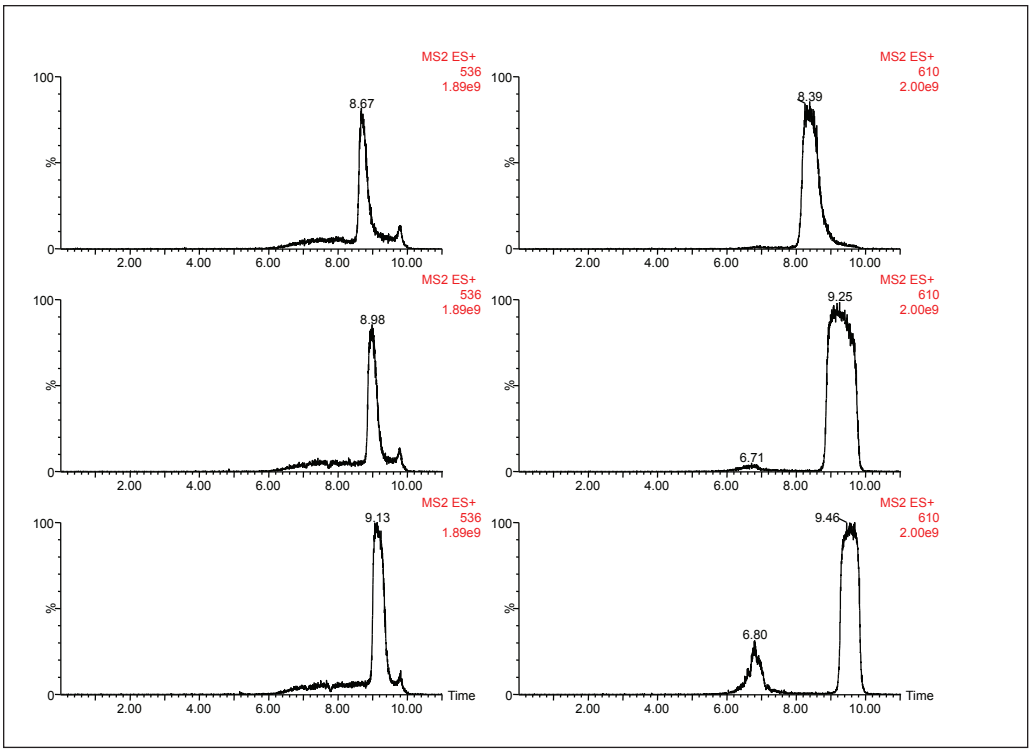

Figure 1d. Low bleed silicone septa with an extracted chromatography 338.6 ion shows a distorted signal in methanol and a sharp tailing peak in acetonitrile suggesting intermediate polarity. The mass at 609.8 shows little signal in methanol suggesting low solubility in more polar solvents and strong signals in acetonitrile and acetone, suggesting an affinity for intermediate solvent strength.

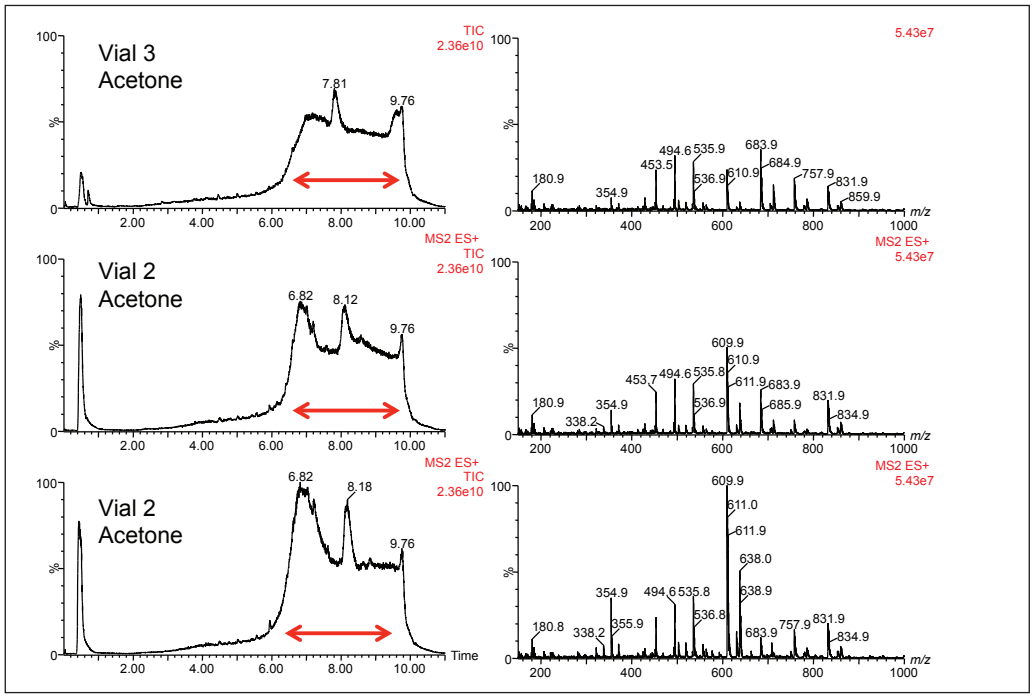

Figure 1e. The chromatograms on the left are total ion current chromatograms (TIC's) in an acetonitrile gradient. Three, 2-mL vial brands soaked in acetone for 30 minutes (5 replicates for each vial brand), covered with aluminum foil and the extract was run on an Open Architecture UPLC System with 2D-LC Technology and at-column dilution. The spectra on the right are the combined spectrum within the red arrows of the chromatogram on the left. The rising baseline is typical for acetonitrile at increasing organic level.

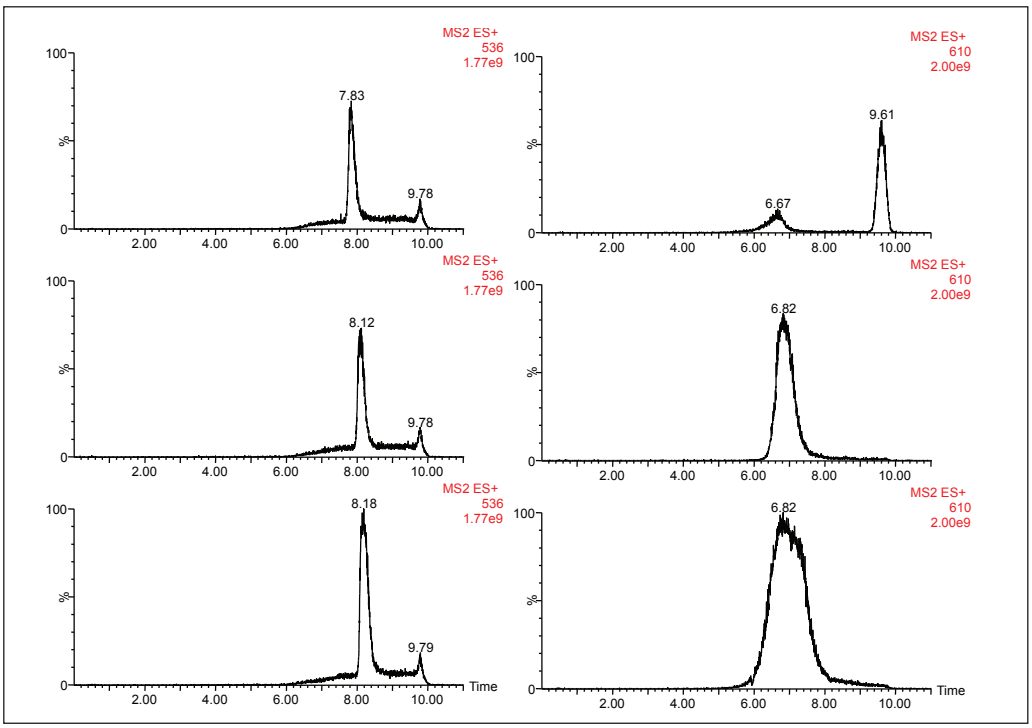

Figure 1f. Two ions were extracted from the TIC, 536 and 610 from figure 1E from the acetone extract. 536 ion shows up later at a similar retention time and a Gaussian peak shape in all three brands of vials. The 610 ion shows also with different retentions in vial 3 than in vials 1 and 2, suggesting vials have same parent mass at 610, but they could be different entities.

with fragmentation experiments. With the acetonitrile extract, the 536 ion shows up at a later and relatively same retention time with a gaussian peak shape in all three vial traces. The same scenario is seen with the acetone extract for all three vials. The 609 ion was selected because of it was previously detected in all silicon septum leaching experiments. The 609 ion is part of a silicon distribution (see Figure 1A). As expected, the 609 shows up as a later eluter and highly soluble in methanol, acetonitrile, and acetone.In this case, further MS/MS characterization can add additional information with fragmentation experiments. With the acetonitrile extract, the 536 ion shows up at a later and relatively same retention time with a gaussian peak shape in all three vial traces. The same scenario is seen with the acetone extract for all three vials. The 609 ion was selected because of it was previously detected in all silicon septum leaching experiments. The 609 ion is part of a silicon distribution (see Figure 1A). As expected, the 609 shows up as a later eluter and highly soluble in methanol, acetonitrile, and acetone.

CONCLUSIONS

The techniques demonstrated in the chapters of this paper can be used to screen for acceptable packaging materials or study process of producing cleaner packaging material. Best materials can be selected to protect the package contents without compromising the quality of the contents. Alternatively, this technique can be used to study and improve the process of producing the packaging. Samples can be taken at different process points and conditions to study and control the blooming and leaching of ions from the materials. This is a cost-effective technique to screen for many solvents and process conditions.

References.

1. Mallet, C., Multi-Dimension Chromatography Compendium: Trap and Elute vs AT-column dilution, Waters Corporation, 2014. 720005339en.
2. Mallet, C. Why are LC Vials Showing Ghost Peaks with the New Generation of Mass Spectrometers? Chapter 1 - Leachables from Silicon Septum: Infusion Analysis. Waters Corporation, 2014. 720005335en.

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