MULTI-DIMENSIONAL CHROMATOGRAPHY COMPENDIUM

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TRAP AND ELUTE VS AT-COLUMN DILUTION

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Hyphenated systems, or multi-dimensional chromatography, is not a new topic; the first hyphenated system (GC-MS) was introduced in mid 1960s. The major benefit of hyphenated systems is the capability of combining key functions from each dimension, which leads to enhanced performance for solving complex analyses. The analysis of a complex samples is the most perceived usage of hyphenated systems, which stems from its increased peak capacity or separation power. Although the high performance of hyphenated systems provides a clear advantage over single-dimensional solutions, hyphenated solutions have been viewed as academic curiosities and too complex for routine application, and are sometimes perceived as being too difficult to operate from a technical perspective. However, with today's computer technologies, hyphenated systems are now available under full automation control. Custom configurations can be built to provide a cost-effective solution for complex sample preparation protocols.

Hyphenated systems provide a clear advantage over single-dimensional solutions. With full automation control, multi-dimensional solutions can be quickly configured to provide several key benefits for difficult applications. The upgrade of a single-dimensional chromatography unit to a two-dimensional (e.g., trap and elute mode one) is a relatively simple process. As show in Figure 1, with the addition of an extra pump, valve, and trapping column, the trap and elute configuration offers the option to inject large sample volume (up to 1 mL) regardless of the sample solvent composition, thus eliminating the need for solvent exchange. This feature is achieved by using a short column packed with a large particle sorbent for the first dimension.

The trapping column (D1) is not designed to be operated under high chromatographic resolution. From the van Deemter equation, the resolution performance of a chromatography process has an intricate link to the particle diameter of the packing material. From a practical aspect, a column packed with small particle size will produce higher resolution performance (Rs) and separation power (Height Equivalent to Theoretical Plate or HETP) with the consequence of creating higher back pressure.

The trap and elute configuration combines both features, by using two distinct column chemistries, each with their optimum operating conditions. The high-resolution column is positioned on the back end of the configuration and directly connected to a detector. The trap column, via a switching valve, is operated in load or elute mode. In load mode, the trap column is now directly connected to an injection valve driven by a loading pump. Since the trap column is packed with large particles (>10 µm), analytes are captured on the trapping material by using optimized flow rates, chemistry, and additives during the loading phase. The main objectives during this step is to use conditions with the highest k' for maximum peak trapping and minimizing potential breakthrough effects. Once the analytes of interest are trapped on the sorbent, the trap column is re-positioned into the elution stream (backflush elution) for analysis.

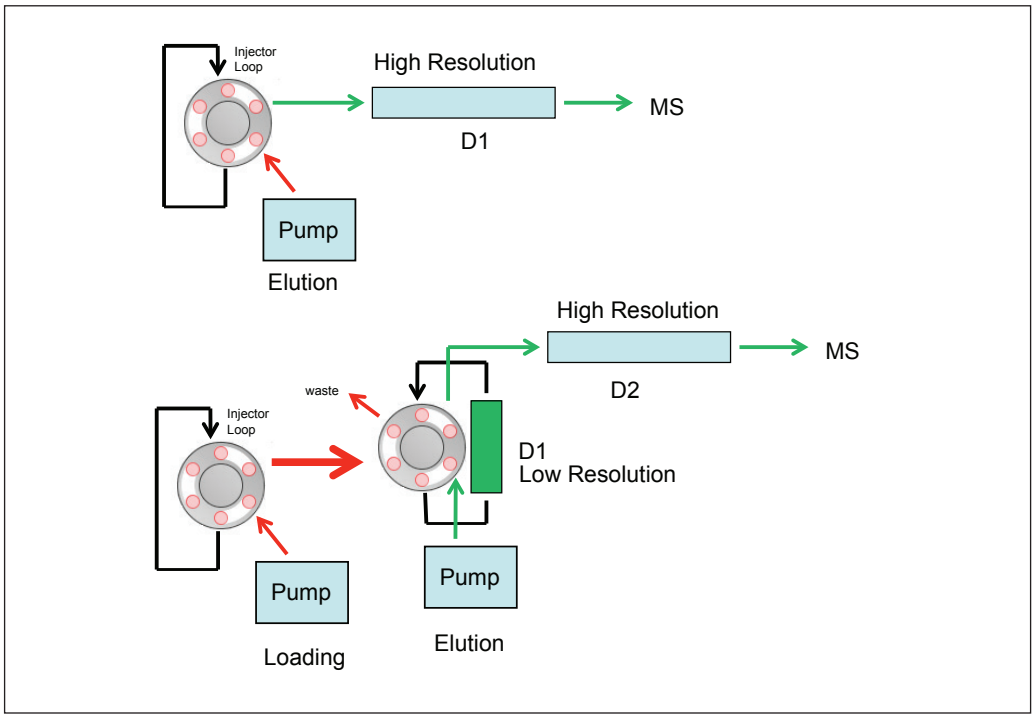


Figure 1. 1D vs 2D configuration with single stream loading.

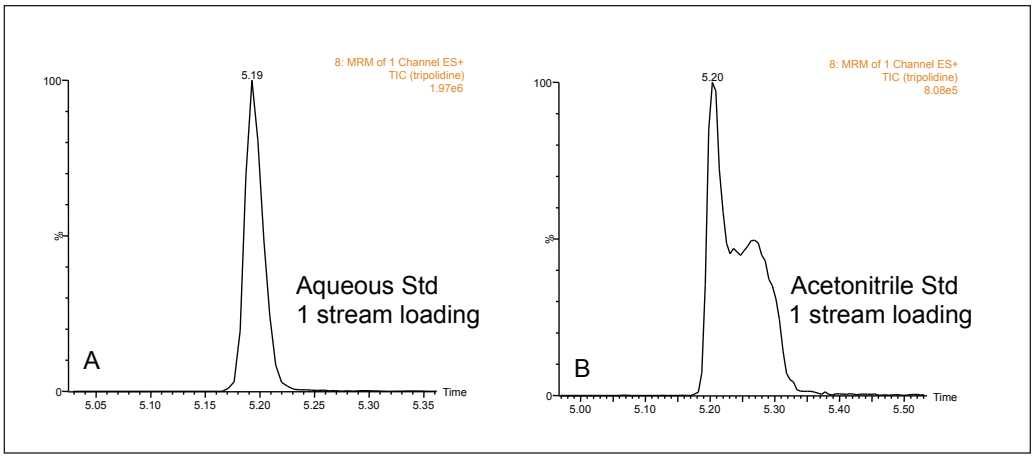


Figure 2. Aqueous vs organic extract using 2D single stream loading.

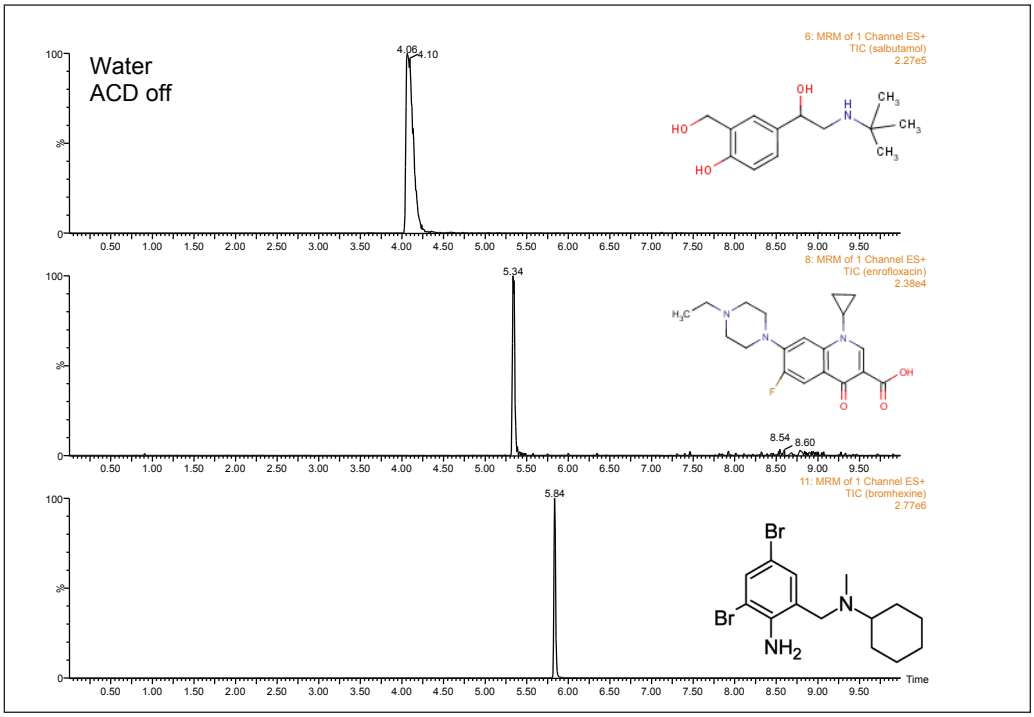


Figure 3. Early-, mid-, and late-eluter in water using 2D single stream loading.

As presented in Figure 2A, an aqueous extract is loaded onto a trap column using a single stream loading. As expected, the profile of the analyte shows a Gaussian distribution. However, the peak is distorted when the same analyte is loaded onto the trap column but in this instance the analyte was dissolved in acetonitrile (Figure 2B). As can be seen, the peak shape shows a distorted profile. The peak splitting profile is the most common behavior when loading k' is not optimized properly. Furthermore, a shoulder peak appears and cause an increase peak width. In this instance, the peak distortion occurs during the loading phase on the trapping column. The loading pump moves the content of the injection loop toward the trapping column, and since the loop volume is made up of 100% organic solvent, the analyte's affinity for the mobile phase will have a high value (low k'). Therefore, in these loading conditions, the analyte's transfer rate from the mobile phase to the stationary will create a dual focusing zone during the injection step. In order to obtain Gaussian peak shape, the analyte must be focused into a tight and narrow band at the column's head. The split peak profile seen in Figure 2B suggests that the acetonitrile percentage is still too high during the injection step, and a significant amount of the analyte travels further into the sorbent bed.

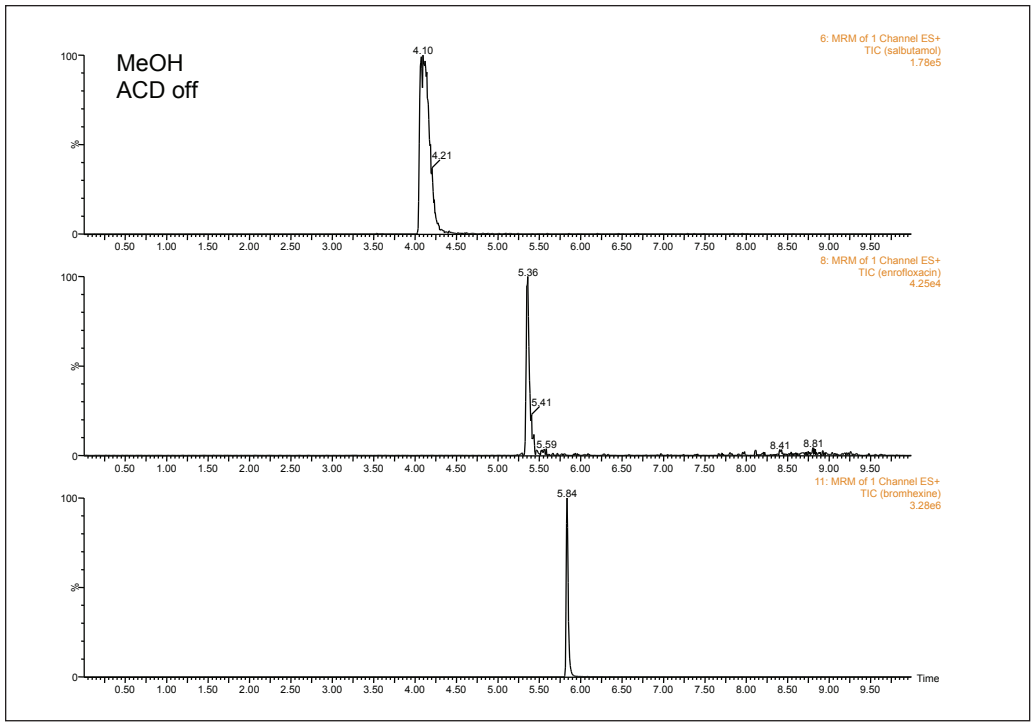


Figure 4. Early-, mid-, and late-eluter in methanol using 2D single stream loading.

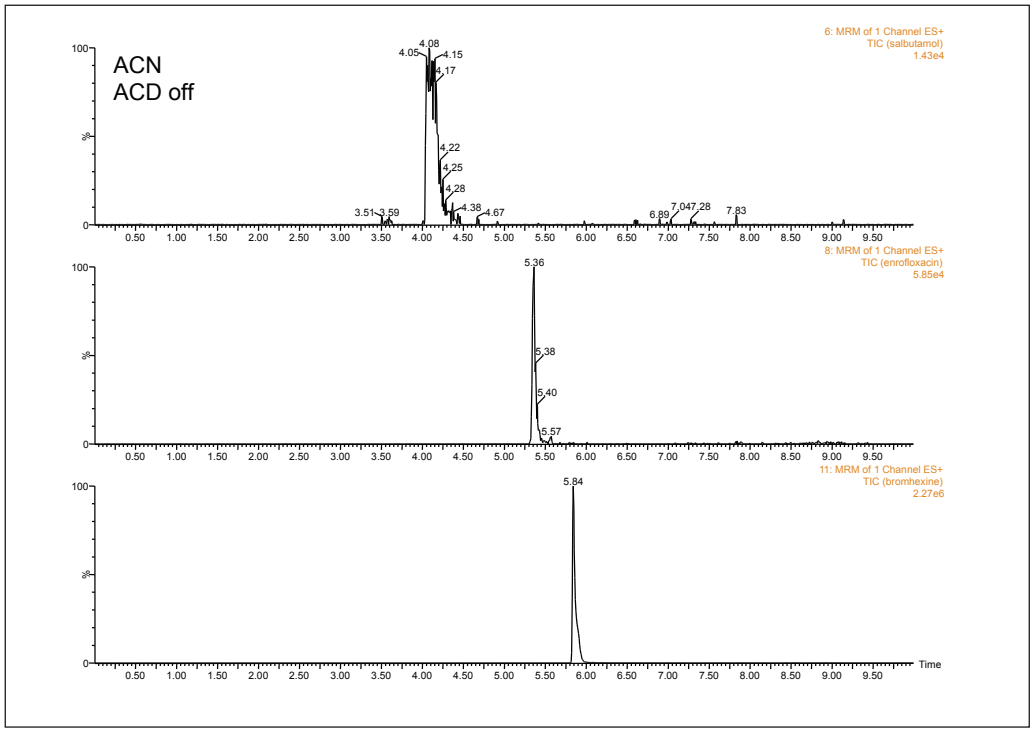


Figure 5. Early-, mid-, and late-eluter in acetonitrile using 2D single stream loading.

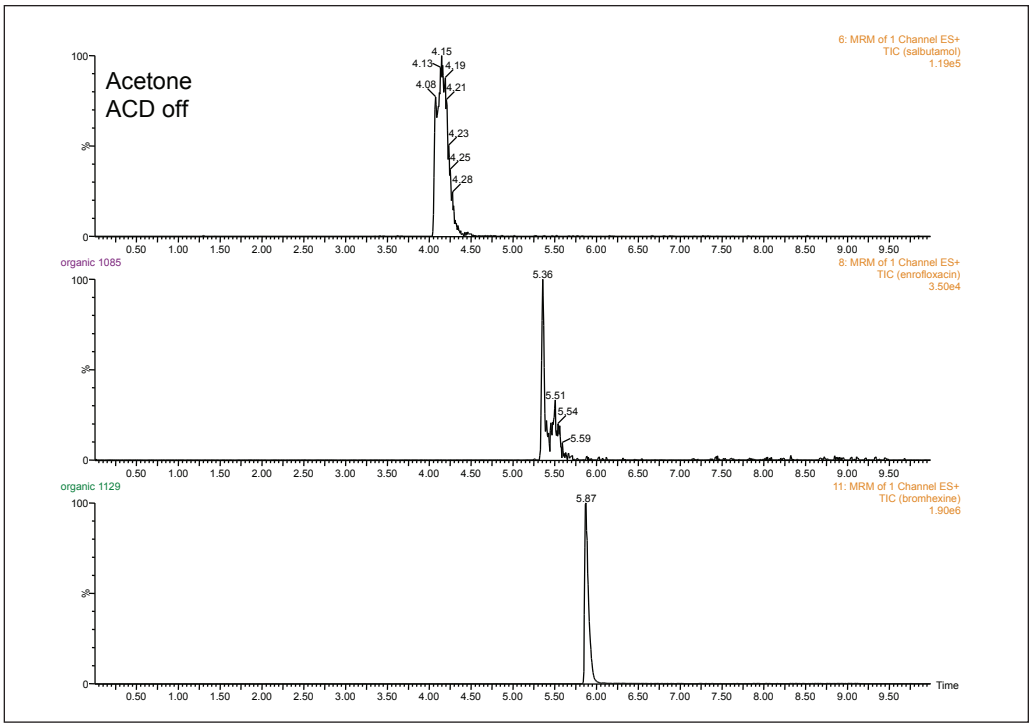
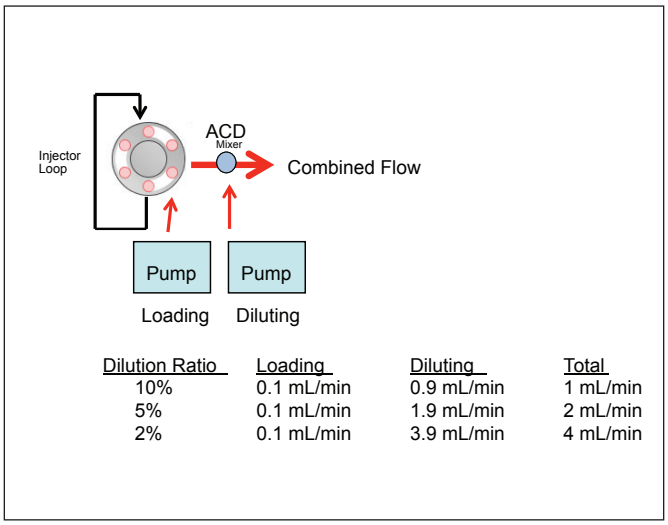


Figure 6. Early, mid, and late eluter in acetone using 2D single stream loading.

Other chromatography profiles are also tell-tale signs of non-optimized loading conditions. The chromatograms in Figure 3 show early-, middle-, and late-eluting peaks with a single stream aqueous loading using an aqueous sample matrix. As it can be seen, all three analytes show excellent Gaussian distribution. However, the early elute shows a wide peak width. This chromatography profile is linked to poor refocusing on the high resolution dimension. In some applications, a target analyte can have a reduced solubility or insoluble in water. In this situation, the loading of an extract dissolved into an aqueous soluble organic will produce peak distortion, as seen in Figures 4 (methanol), 5 (acetonitrile), and 6 (acetone). Since k' is a measurement of an analyte's affinity for a stationary phase and the mobile phase, a target analyte's k' can be affected by its solubility in various organic solvents. As demonstrated for the early-eluting peak, Salbutamol, the analyte shows high e5 signal for the aqueous (Figure 3), methanol (Figure 4), and acetone (Figure 6) results. The chromatogram in Figure 5 with the acetonitrile matrix shows a low e4 signal, suggesting complete breakthrough during loading.

The trap and elute configuration is limited to aqueous extracts only. To achieve effective focusing for organic extracts, the challenge is to reduce the organic percentage by diluting with aqueous (see Figure 7). The dilution is performed by combining two flow streams: one stream is connected to the injector port (loading stream), and the second stream (dilutor stream) is connected after the injector with a low volume mixer (3-way). The flow rates of each stream are set to produce a desired dilution ratio. As shown in Figure 8, two options are available, using a two- or three-pump design. Both designs offer similar analytical performance, however the three pumps approach has a higher dilution ratio capability. The two streams design utilizes a split loading stream to create a dilution effect for the injection volume before reaching the trapping column. This option now offers the possibility to inject both aqueous and organic extract. This configuration effectively utilizes at-column dilution, the benefits of which are shown in Figure 9.



Dilution Ratio	Loading	Diluting	Total
10%	0.1 mL/min	0.9 mL/min	1 mL/min
5%	0.1 mL/min	1.9 mL/min	2 mL/min
2%	0.1 mL/min	3.9 mL/min	4 mL/min

Figure 7. Leachable ion distribution from a silicon cap soaked in methanol.

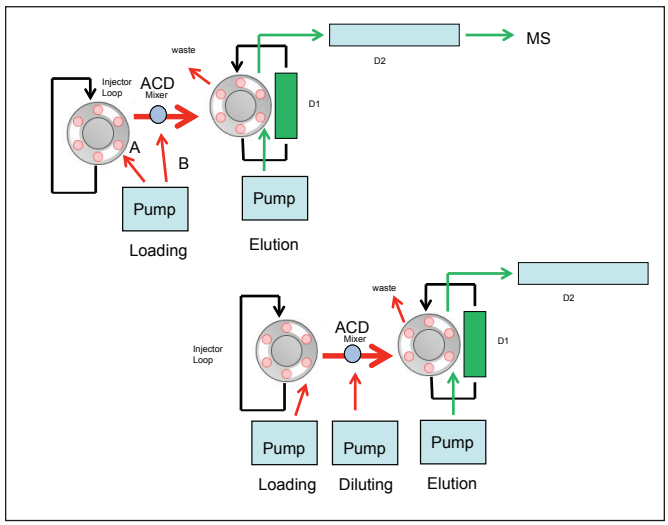


Figure 8. Current extraction protocol and techniques for leachable experiments.

The at-column dilution reduced the amount of organic solvent to an optimum ratio to ensure high k' during peak focusing. As a result, both aqueous and organic injections produce a Gaussian peak shape.

Figure 10 shows the retention profile of three analytes in aqueous (low-, intermediate-, and late-eluting peaks) with the at-column dilution set at 5%. The peak distribution shows a typical Gaussian shape for all three analytes. As expected, since there is no organic solvent in the sample, peaks are showing no tailing or split peak effect. However, the intermediate analyte shows a weak response in comparison to the analytes. In Figure 11, the sample analytes are injected with the same loading and eluting conditions, with one exception being that the sample was dissolved in 100% methanol. As it can be seen, the signal for the intermediate and late-eluting peaks shows increase levels when compared to the aqueous sample (see Figure 10). This can be explained by either a higher solubility in methanol than in water or a reduction of ion exchange retention with the glass vial surface.

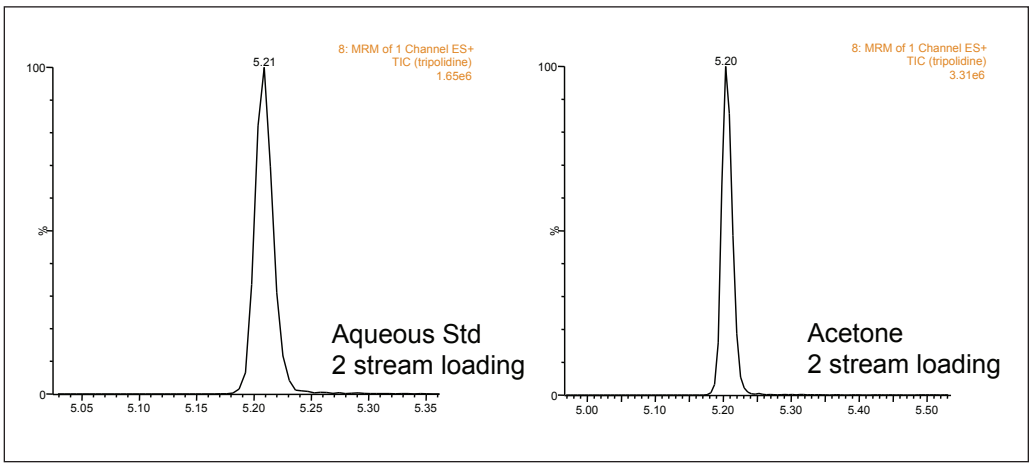


Figure 9. Aqueous vs organic extract using 2D with at-column dilution.

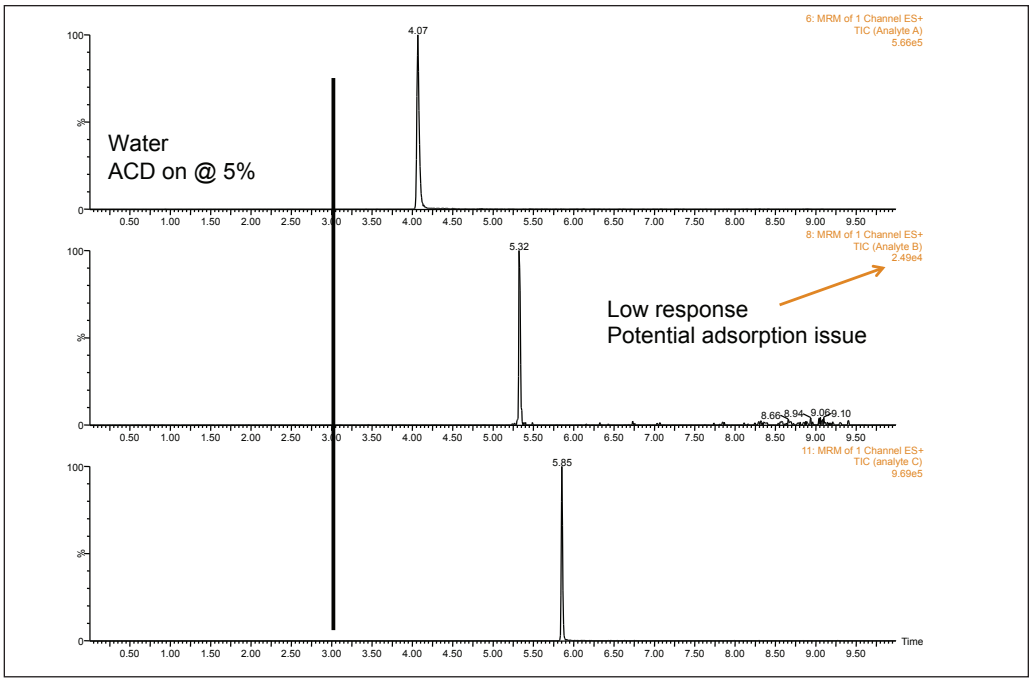


Figure 10. Early-, mid-, and late-eluter in water using 2D with at-column dilution (5%).

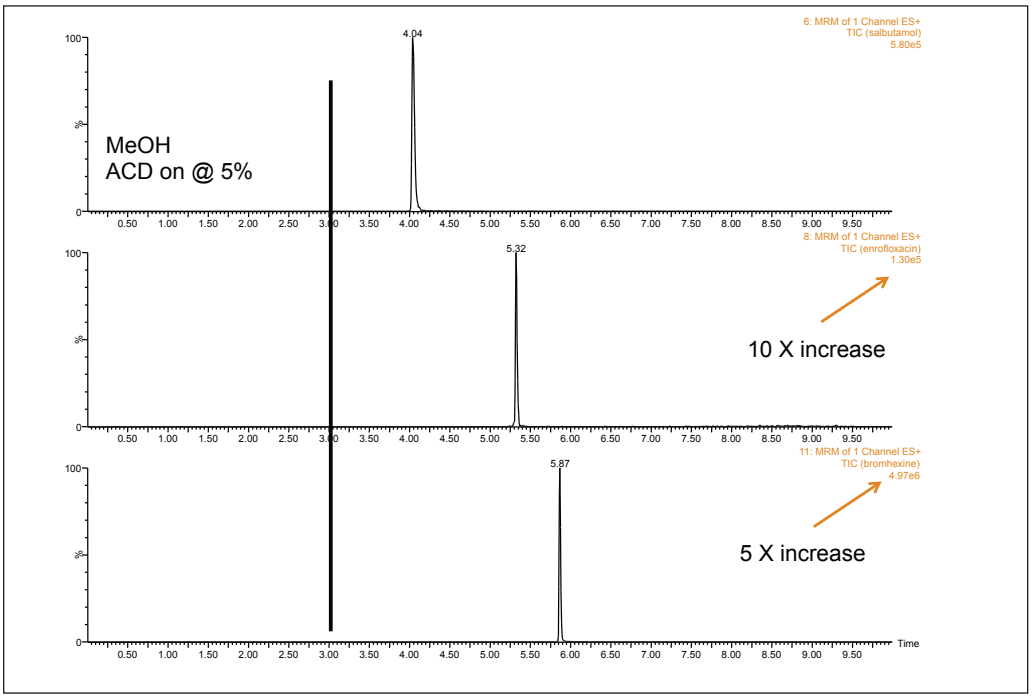


Figure 11. Early-, mid-, and late-eluter in methanol using 2D with at-column dilution (5%).

By decreasing the organic solvent elution strength, Figures 12 and 13, show the result when a sample is dissolved in acetonitrile and acetone, respectively. In these cases, the polarities of the solvents are lower than methanol and can cause peak distortion during peak re-focusing. As it can be seen, the early-eluting peak was drastically affected by a 50% signal drop and the appearance of a shoulder peak. The effect is more predominant if the sample is dissolved in acetone (intermediate polarity) and affects both the early- and intermediate-eluting peaks. The loading conditions require a lower dilution ratio of organic solvent. A three-pump configuration was selected for its high-performance capability (Figure 14) and produced excellent peak shape for the three

analytes in acetone using a 2% at-column dilution factor. The only difference between a 5% and 2% is loading time. As it can be seen, with a 5% loading dilution, the loading time required was set at 3 min for the entire injection loop content to be loaded onto the trapping column. By reducing the loading stream flow rate, the loading time must therefore be extended to achieve optimum focusing on the trapping column.

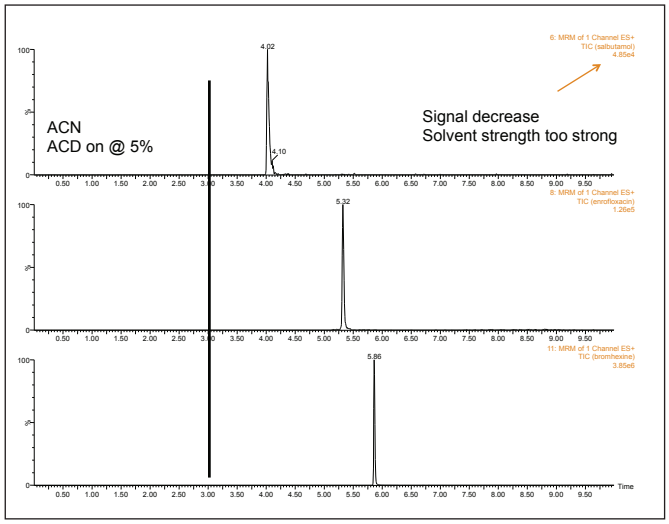


Figure 12. Early-, mid-, and late-elute in acetonitrile using 2D with at-column dilution (5%).

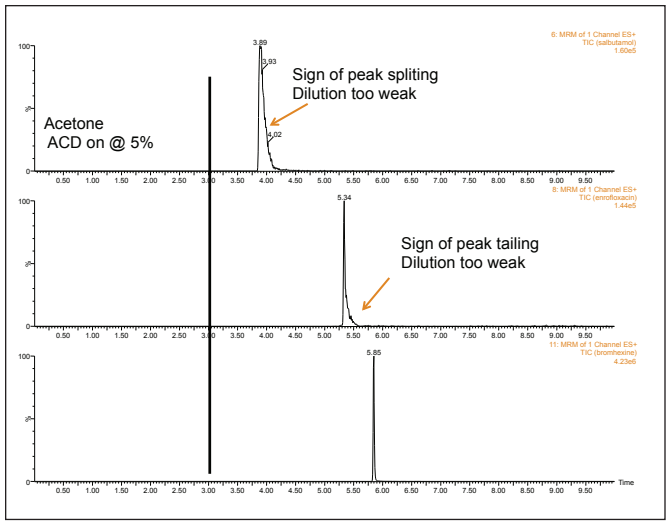


Figure 13. Early-, mid-, and late-eluter in acetone using 2D with at-column dilution (5%).

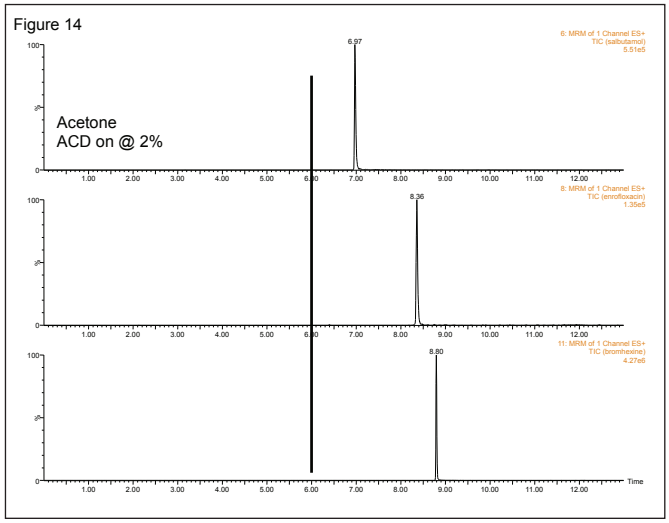


Figure 14. Early-, mid-, and late-eluter in acetone using 2D with at-column dilution (2%).

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