MULTI-DIMENSION CHROMATOGRAPHY COMPENDIUM

Loop vs. Trap Injection

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INTRODUCTION

Since the discovery of the chromatography concept by Twsett in the early 1900's, the separation technique has certainly seen major innovations over several decades. As new technology emerges, new options become available thus bringing effective performance for difficult applications. The concept of hyphenated systems or multi-dimensional chromatography was designed for solving complex analyses. The peak capacity or separation power can be increased by combining several separation dimensions (most instances utilize two) each using optimized conditions for maximum resolution. The main challenge is the transfer of closely resolved analytes from the primary resolving dimension (PRD) to the secondary resolving dimension (SRD). The transfer between analytical columns is not a trivial exercise and can lead to poor performance. As seen in Figure 1, when using a serial connection (loop injection) between two analytical columns, a pair of well resolved analytes in the PRD can co-elute in the SRD, thus creating an overall neutral effect. Therefore, to solve this issue, the concept of decoupling time by trap injection, leads to an orthogonal separation performance, in which the overall separation is un-affected by co-elution effects from either dimension (see Figure 2). In comprehensive chromatography mode (serial connection), the chromatography conditions in the PRD are usually set with slow elution conditions over a long period of time. The resulting chromatogram is cut in multiple fractions by two oscillating loops for refocusing and separation on the SRD. With this configuration, the SRD is usually set in fast elution mode with rapid equilibration and high flow rate. This time constraint is linked to the fill rate of the loop injection cycle which in most application varies between 1 and 2 minutes cycle for loop transfer, peak re-focusing, separation, and column equilibration. This time limitation can lead to poor separation and resolution.

RESULTS AND DISCUSSION

Resolution, in its simplest terms, is the characterization of the separation between two peaks, and can vary dependent on the slope of the % change of the mobile phase conditions. The work will start with a chromatogram, as seen in Figure 3, showing a separation of several analytes using a low flow rate elution over a 5 minute gradient elution. The chromatography conditions and column size for the PRD are typical of comprehensive chromatography. In this instance, for timing reasons, the gradient was set at 5 minutes instead of the typical 3–4 hours, but keeping similar flow rate. A mixture of several analytes was carefully selected to ensure elution in the early, intermediate and late portion of the gradient. The chromatogram shows three regions of interest that will be use for evaluation between loop and trap injection. The area selected (A, B, and C) show two pair with co-elutions and a pair with baseline resolution.

The comprehensive chromatography separation was set with a 50 µL injection loop between the PRD and the SRD. The PRD flow rate set at 0.1 mL/min with a 0.5 minute cycle (30 seconds cut for the SRD. The SRD column I.D. set at 2.1 x 50 mm with a 0.5 mL/min

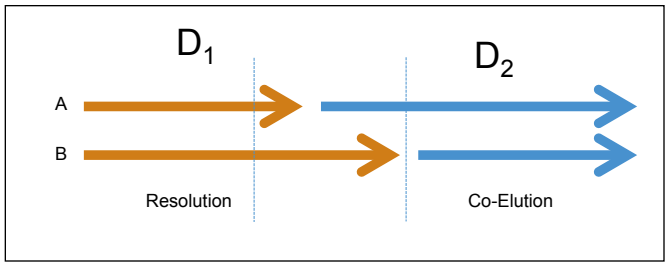

Figure 1. Serial resolution.

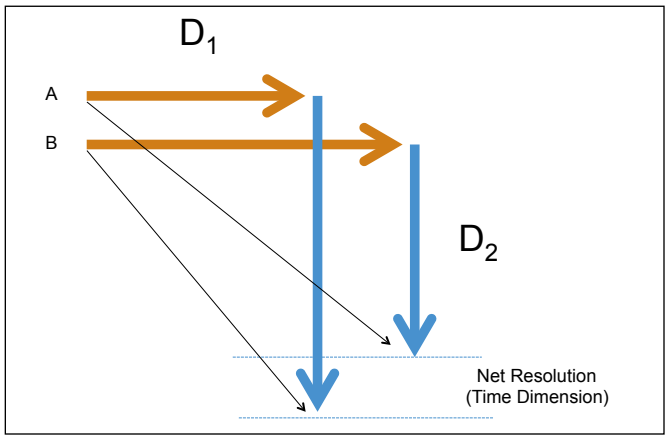

Figure 2. Orthogonal resolution.

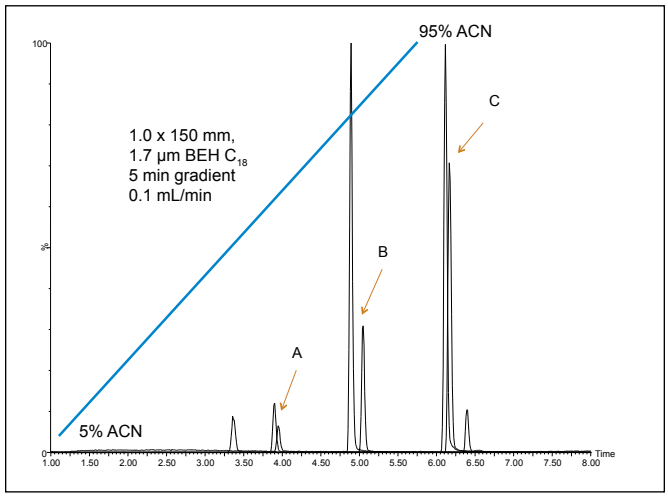

Figure 3. 1D chromatogram separation.

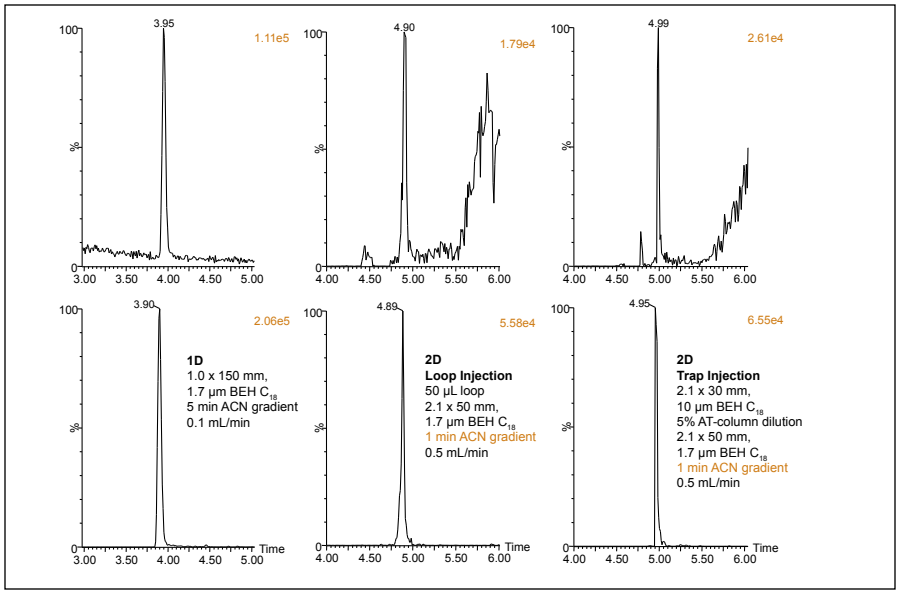

Figure 4A. Section A loop injection vs. trap injection with 1 minute elution cycle.

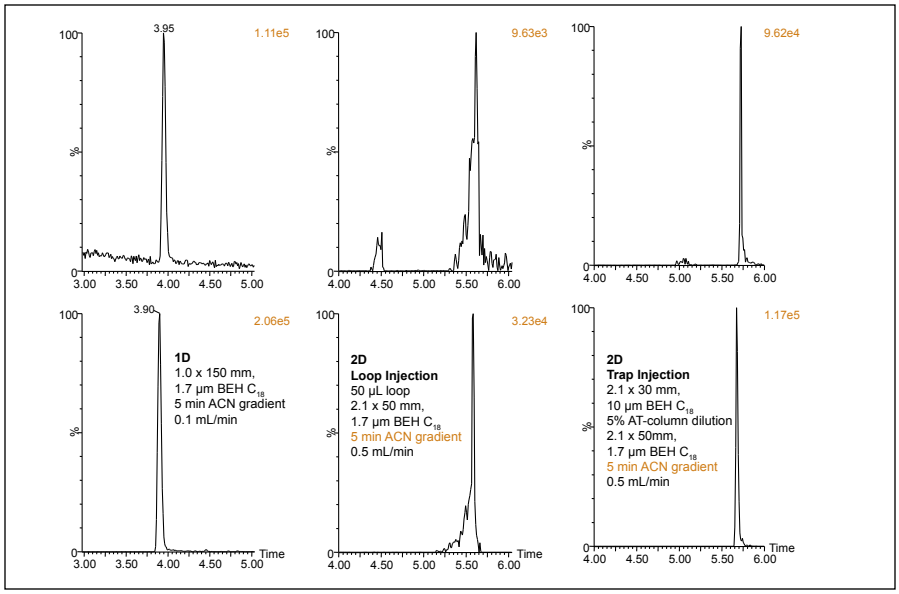

Figure 4B. Section A loop injection vs. trap injection with 5 minutes elution cycle.

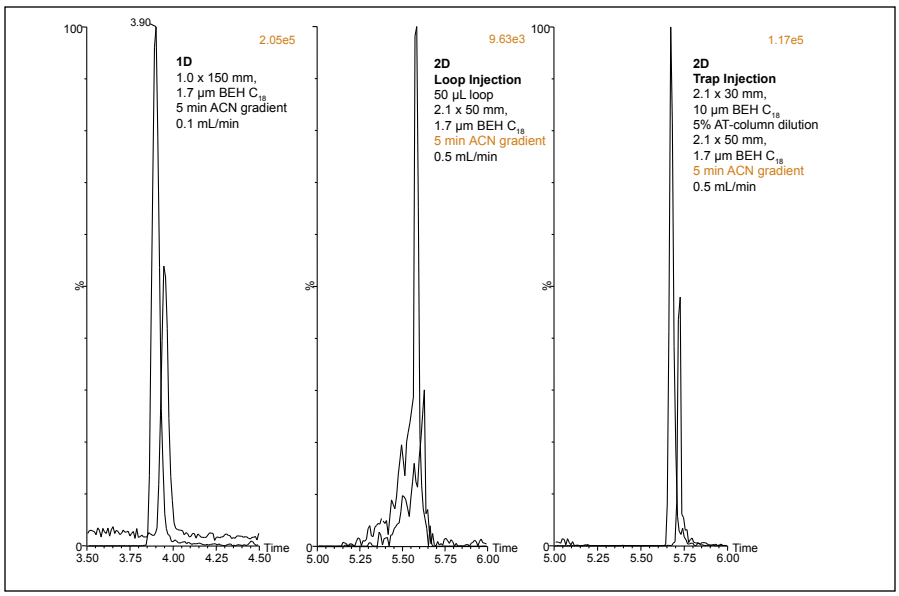

Figure 4C. Section A superimposed chromatogram.

flow rate which gives a calculated 10:1 dilution ratio. Dilution ratios for comprehensive chromatography are typically set at 50:1 or higher, thus the need for larger I.D. (4.6 x 50 mm), particle size (>5 µm) and flow rate in the 2–3 mL/min range. When operating with those conditions, a flow splitter will be required for MS detection (20:1). For the time decoupled option, the 50 µL loop was simply replaced with a 2.1 x 30 mm trap with the same packing material as the SRD with larger particle size (>10 µm). The same 0.5 minute cycle was kept for comparison purpose. AT-column dilution was set at 5% for optimum re-focusing. The same elution conditions were use for both dimensions, with a 1 min and a 5 min elution cycle. The chromatography conditions for the SRD was selected to ensure similar separation performance with the PRD.

In this evaluation, three 30 seconds sections (A, B, and C in Figure 3) of the PRD were excised and re-injected into the SRD using a loop or a trap. The chromatograms in Figure 4A, 4B and 4C show the results for the first pair of analytes (section A). From their respective retention time, the excised volume containing those two analytes has a 40/60 water: acetonitrile ratio. The high organic ratio must be diluted to less than 5% for effective peak refocusing, thus the use of high flow rate values. The chromatogram on the left hand side of Figure 4A shows the chromatographic profile for the 1D separation. As it can be seen, the peaks are showing gaussian distribution with a 50% co-elution. The center chromatograms shows the chromatographic profile for the loop injection using a 1 min elution cycle, while the chromatograms on the right hand side show the same analytical cut using the trapping technique. The chromatographic profile acquired from the loop injection technique show signs of leading which is typical when using fast elution conditions, while the

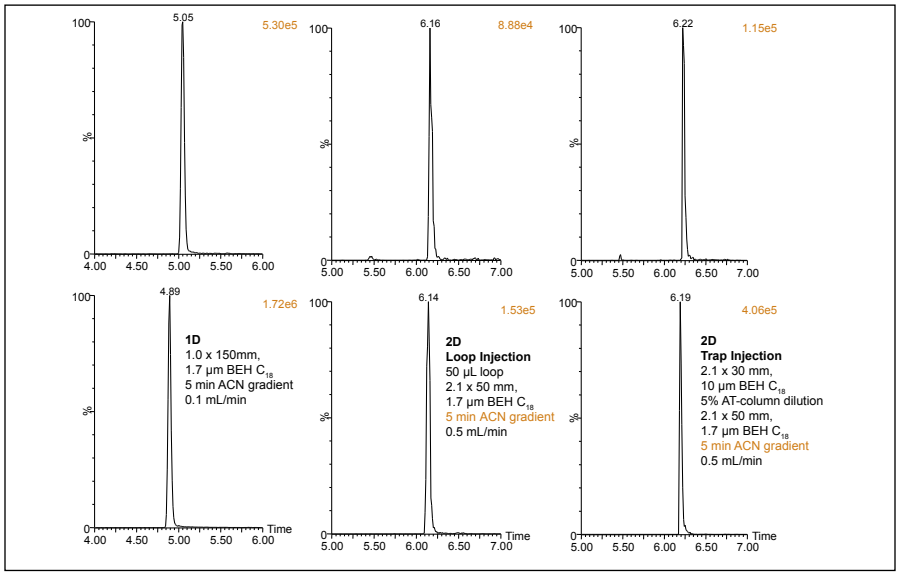

Figure 5A. Section B loop injection vs. trap injection with 1 minute elution cycle

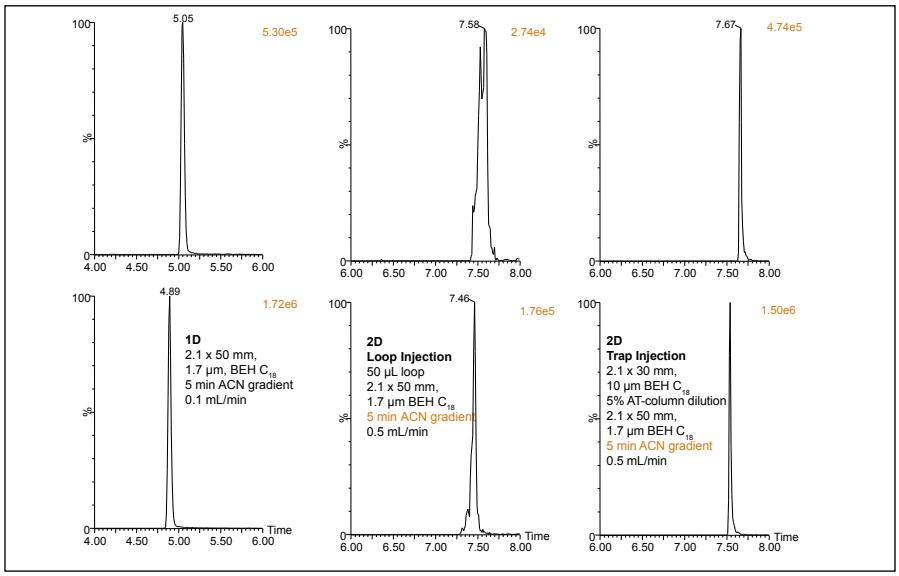

Figure 5B. Section B loop injection vs.trap injection with 5 minutes elution cycle.

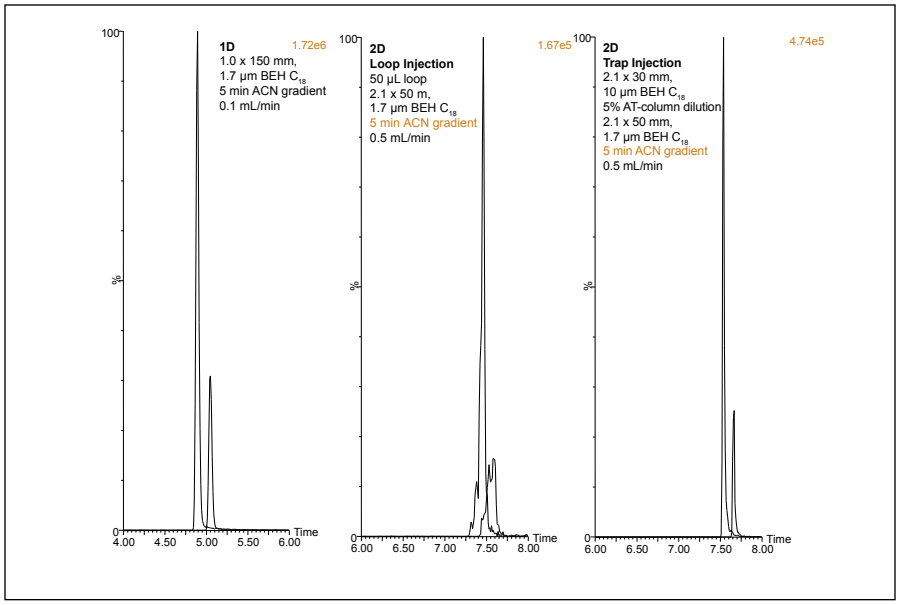

Figure 5C. Section B superimposed chromatogram.

trap injection shows excellent gaussian distribution. In both instances, the mass transfer does not match values recorded with the 1D chromatograms, simply because mass transfer conditions are too fast. The chromatograms in Figure 4B shows the results with a 5 min elution cycle, which clearly show peak distortion for both analytes when using the loop injection technique versus the trap injection with excellent peak distribution and matching area counts when compared the 1D chromatogram. In Figure 4C, the chromatograms are super-imposed to visualize the resolution lost or gain between the loop and trap injection mode. As it can be seen, the resolution is completely lost with the loop injection (chromatograms in center), but shows a noticeable improvement with the trap technique (co-elution at 20%).

In section B, the pair of analytes was excised from the PRD at a 25:75 water:acetonitrile ratio. The higher organic percentage will require more time and higher water dilution for effective peak re-focusing. The chromatograms in Figure 5A and 5B show the results for the 1 and 5 minutes cycle, respectively. The results for the loop injection and trap show excellent gaussian peak shape, but with no improvement on resolution and mass transfer. The 5 min cycle give plenty of time for the trap injection mode to show excellent peak shape and mass transfer, while the loop injection show signs of peak distortion. The superimposed chromatograms in Figure 5C shows lost of resolution for the loop injection, and same excellent baseline resolution for the trap injection when compared to the 1D chromatogram. For the analytes in section C,

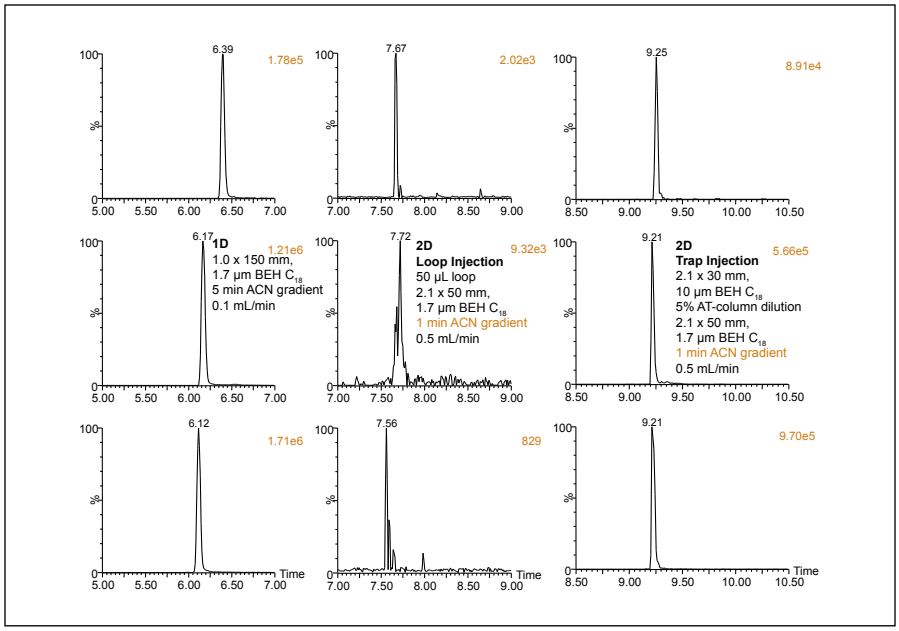

Figure 6A. Section C loop injection vs. trap injection with 1 minute elution cycle.

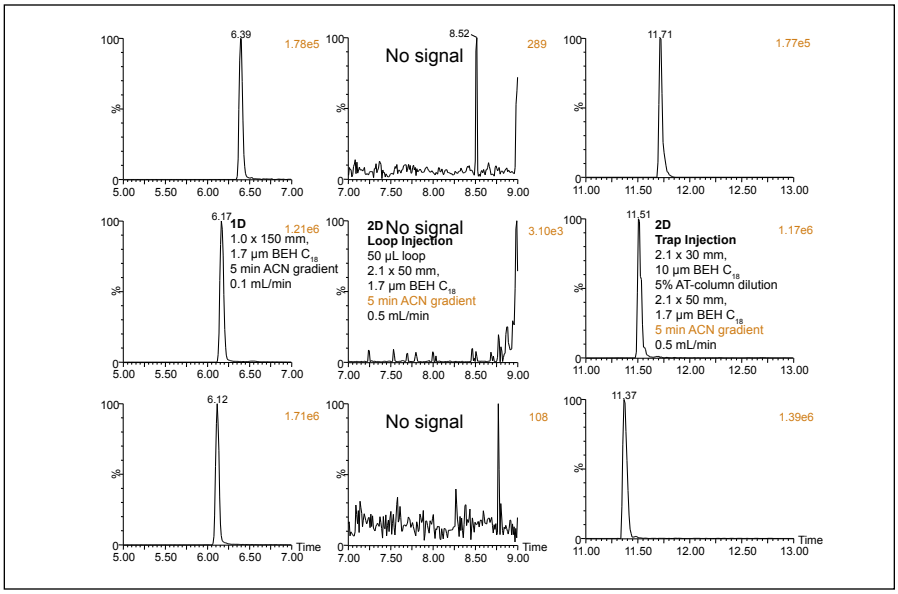

Figure 6B. Section C loop injection vs. trap injection with 5 minutes elution cycle.

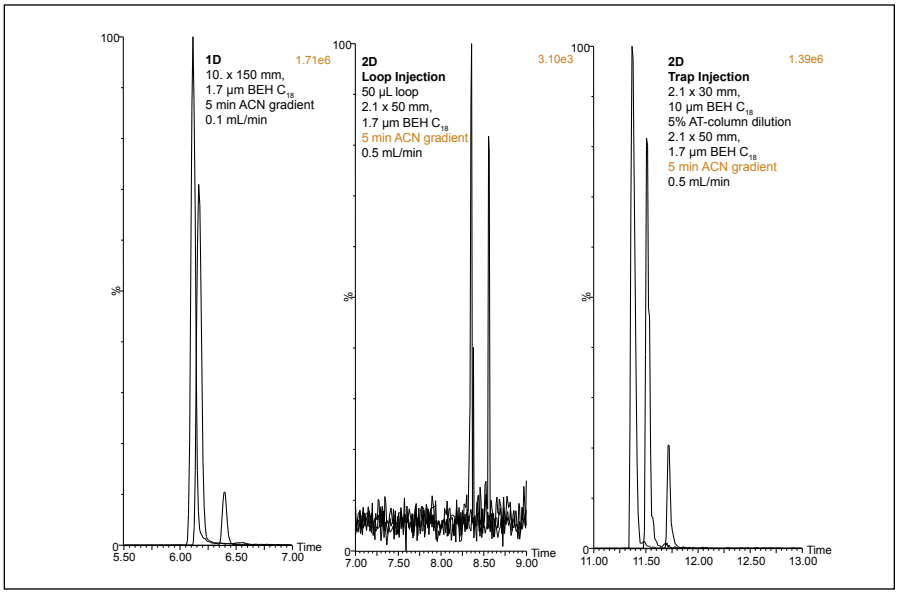

Figure 6C. Section C superimposed chromatogram.

two analytes are co-eluting (50%), with the last analyte well resolved from the other two. The section excised from the 1D chromatogram is composed at 5:95 water:acetonitrile ratio (back end of the gradient from PRD). The chromatogram in Figure 6A and 6B show a total loss of signal of all three analytes when using either the 1 or 5 min loop injection approach, respectively.

The lost of signal is attributed to breakthrough effect when organic percentage are too high during injection. However, for the trap injection, all three analytes show excellent gaussian peak shape with a baseline resolution gain with the 5 min trap injection mode (see Figure 6C).

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