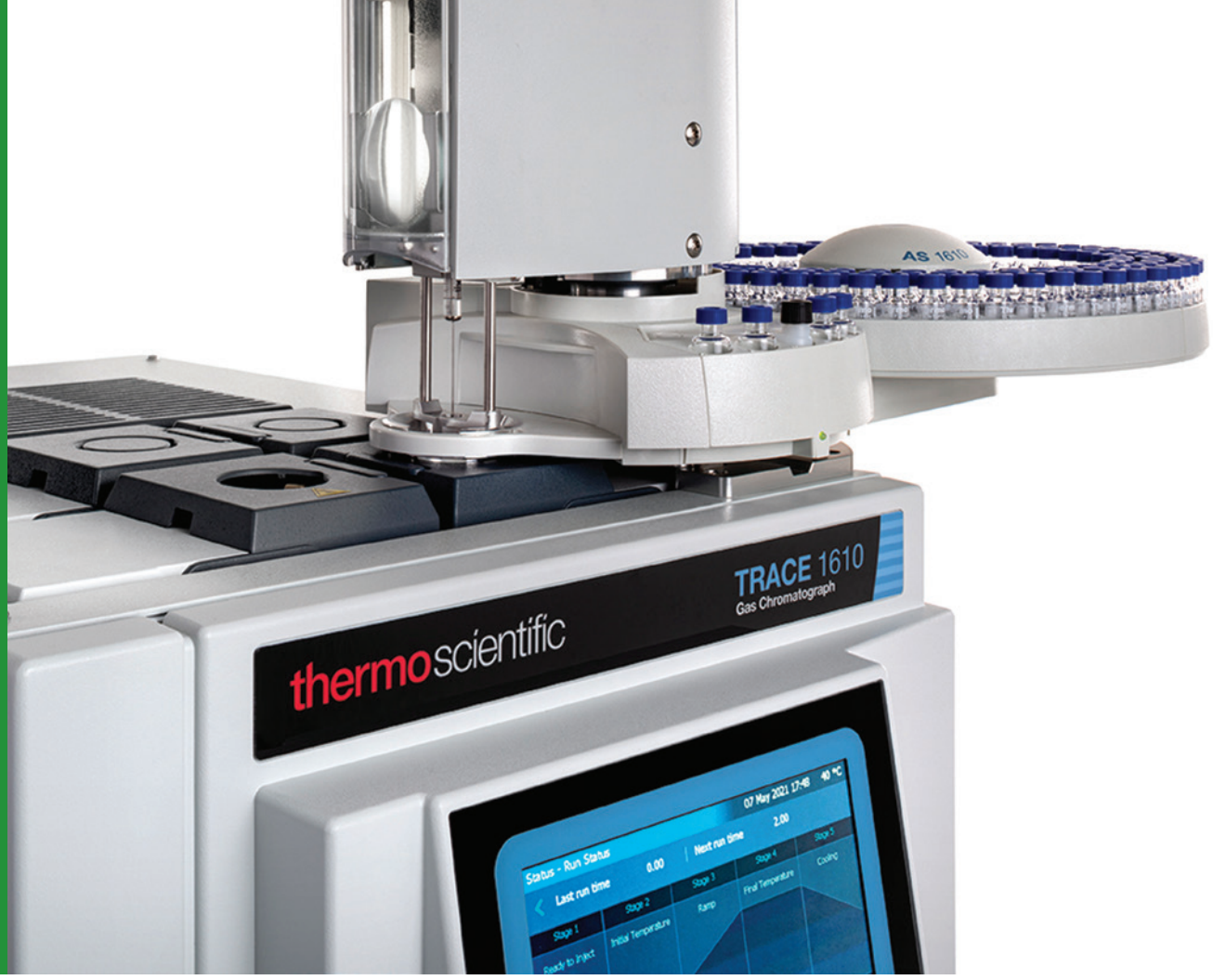
ROBUST AND USER-FRIENDLY BACKFLUSH TECHNOLOGY REDUCES GC MAINTENANCE AND MAXIMIZES LABORATORY THROUGHPUT



Goal

To demonstrate the suitability of the new Thermo Scientific™ Instant Connect PTV-backflush inlet module for analytical testing laboratories wanting to increase sample throughput and improve method robustness.

Introduction

Analytical testing laboratories require the capability to analyze a large number of samples every day, for both screening and quantification of specific target analytes. However, these analytes are often present in complex matrices, such as food or fuel, that contain many other compounds that can adversely affect the routine maintenance interval, reducing the overall laboratory throughput and increasing the cost per sample.

To overcome the detrimental effects of the matrix on the instrumentation during these analyses, various steps are taken, often including rigorous sample clean-up procedures like solid phase extraction (SPE). However, SPE can only reduce the matrix and some unwanted, high boiling compounds often remain in the samples. Also, for some samples, clean-up cannot be used at all, so an alternative approach to improving method robustness is required.

An alternative method is to use GC column backflushing, which works by reversing the flow of carrier gas through the capillary column at a specific time point defined by the analyst, usually after the last compound of interest has eluted.

However, backflush technology has historically been difficult to adopt and use as it is not always user friendly or robust, and usually requires an auxiliary gas, adding complexity to the GC configuration. This is particularly problematic for analytical testing laboratories where robustness and sample throughput are critical to business success, and where analysts are required to be able to install and use the system quickly with minimal training.

Here we describe a novel backflush injection device, with a robust design, that is easy to implement and use with the Thermo Scientific™ TRACE™ 1600 Series GC.

Setup and control

The Thermo Scientific Instant Connect PTV-backflush injector module can be configured as pre-column, post-column, or mid-column backflush with the use of a Thermo Scientific microfluidic device based on SilFlow® technology, which is easily accessible, fitting neatly within the column oven. The pneumatic control is completely integrated in the PTV injector module, removing the complexity and expense of an additional auxiliary gas module, and facilitating easy method setup thanks to the self-adjusting pressure during backflushing. In the pre-column setup (Figure 1A), an injection from the inlet is made onto a pre-column, typically an empty deactivated guard column, before passing into the analytical column. In this setup, the unwanted heavier matrix remains within the pre-column, while the analytes of interest progress to the analytical column. As the backflushing is turned on and the oven temperature increases,

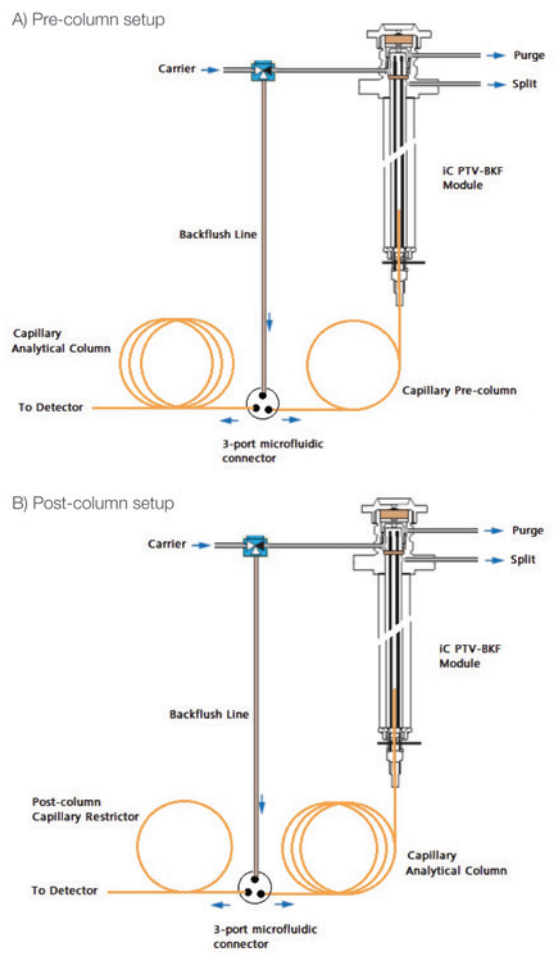


Figure 1. Schematic of the PTV-backflush for the pre-column configuration (A) and the post-column configuration (B)

the matrix contaminants, still in the pre-column, experience a reversal in column flow and are then backflushed through the inlet and vented through the split line. Preventing the high boilers from entering the analytical column has a twofold effect: a shorter run time, and an extended column lifetime with increased instrument uptime between maintenance operations. As an additional benefit, the PTV is heated to a higher temperature during the backflush cleaning step to further reduce the matrix contamination, leaving the inlet extra clean for the next injection.

In the post-column setup (Figure 1B), an injection from the inlet is made directly onto the analytical column, before passing through the backflush microfluidic device and then into a narrow post-column restrictor leading to a detector. In this configuration, when the backflush is turned on, heavier compounds remaining in the analytical column are backflushed to the inlet and expelled again through the split line. This mode backflushes the entire analytical column and is useful for cases when there is matrix contamination across the entire boiling point range, which needs to be separated from the target analytes before backflushing. This mode is often used with mass spectrometry to protect the ion source from unnecessary contamination, further extending uptime and method robustness.

An intermediate configuration is represented by the mid-column backflush, where the empty pre-column is replaced by a short piece of analytical column. This configuration makes it easier to find the right backflush time thanks to the enhanced separation in the pre-column, while allowing convenient replacement of the pre-column as regular maintenance, preserving the analytical column lifetime.

The iConnect PTV-backflush module is easily controlled within the Thermo Scientific™ Chromeleon™ CDS. The timing of the backflush is configured within the instrument method settings for the PTV as part of the cleaning step. The backflush start time is determined by the transfer time in addition to the injection time and the time to reach the transfer temperature (Figure 3). The transfer time is initially adjusted experimentally, usually in 0.5 min increments, and ultimately tightened down to within 0.01 min increments, providing precise and reproducible enough cut times that will split sharp GC-grade peaks in half.

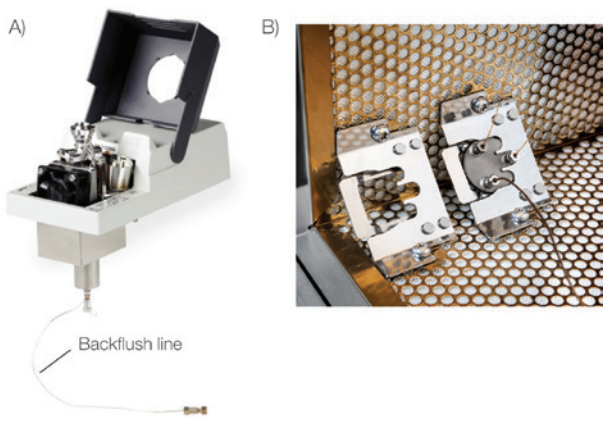


Figure 2. Image showing the iConnect PTV-backflush module (A) and the microfluidic device fitted in the GC oven (B)

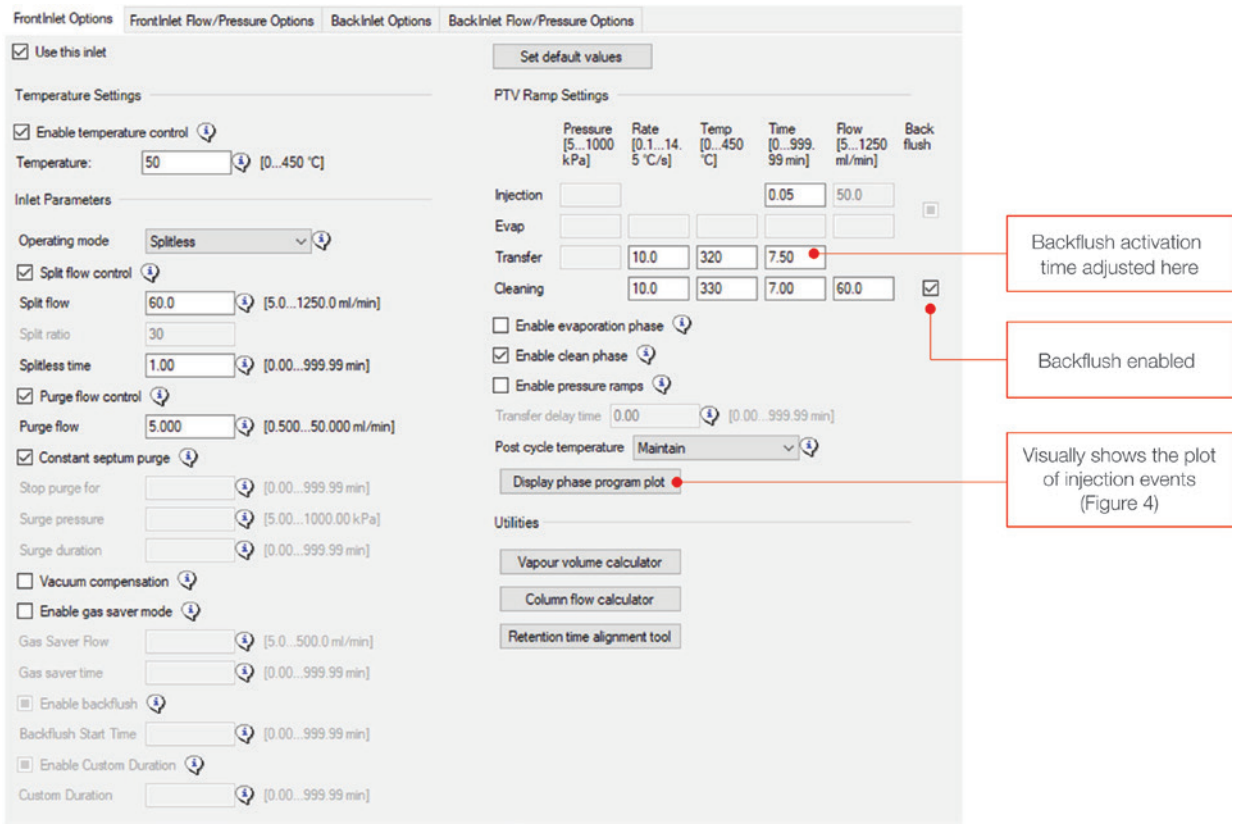


Figure 3. Screenshot of the PTV method page within Chromeleon CDS, with a backflush start time of 8 min

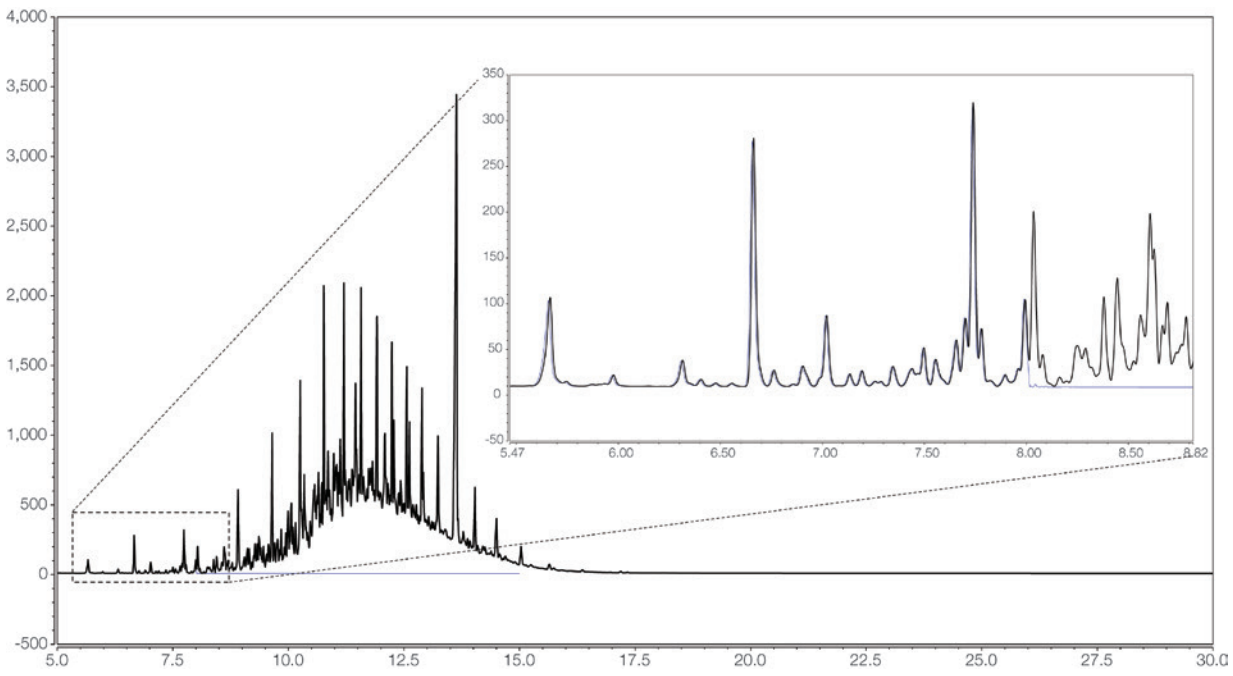


Figure 5. Chromatogram showing a diesel standard spiked with 100 ppm of cyclohexane, toluene, and p-xylene run with backflush (blue) and without backflush (black). The inset shows a zoomed chromatogram around the 8 min region.

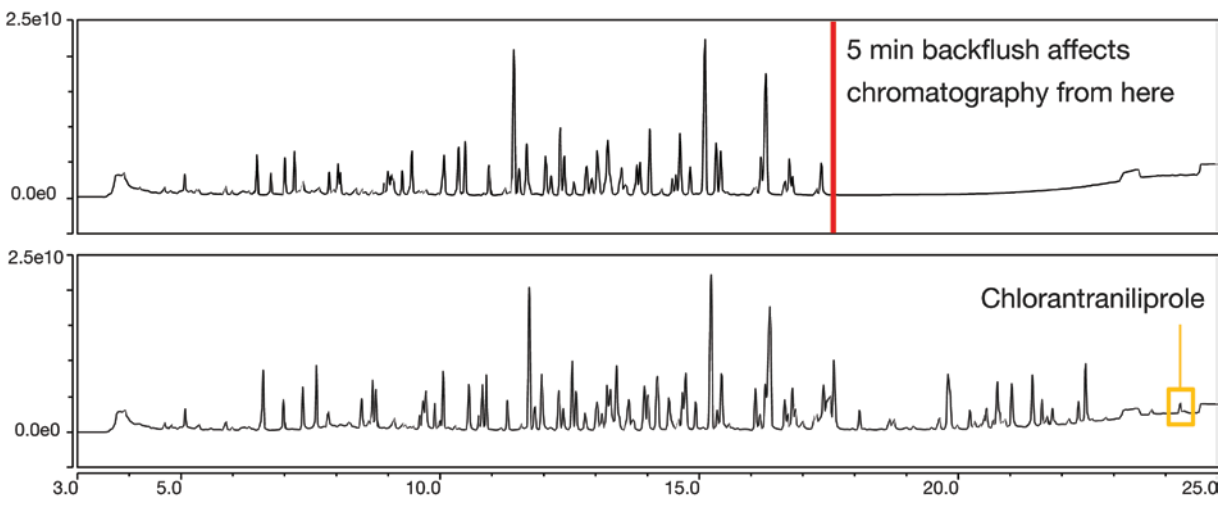


Figure 6. Stacked overlay showing the total ion chromatograms of a mixed pesticide solvent standard with backflush on after 5 min (upper) and after 8 min (lower)

Main configurations

Post-column configuration

When set up in the post-column configuration, the analytical column is connected directly from the PTV injector to the microfluidic device, and a narrow bore restrictor is then connected from the microfluidic device to the detector. In this example, a 30 m x 0.25 mm i.d. x 0.25 µm Thermo Scientific™ TraceGOLD™ TG-1MS analytical column (P/N 26099-1420) was used, coupled to a 1 m x 0.1 mm i.d. uncoated, deactivated transfer line (P/N 60201-393) to connect to the Thermo Scientific iConnect FID. In this configuration, a clean cut at a specific retention time can be easily achieved by turning the backflush on after the last analyte of interest has eluted from the analytical column. This enables a straightforward and quick method setup, while still preventing later eluting compounds from reaching the detector, keeping it cleaner for longer.

This configuration makes precise cuts during the chromatography easy and straightforward. In the example shown in Figure 5, a sample containing 50,000 ppm diesel in dichloromethane (DCM) was spiked with 100 ppm cyclohexane, toluene, and p-xylene and run with and without backflushing. The backflush was enabled at 8 min, and held for an additional 7 min, giving a 15 min run time. A clean cut is made in the chromatography at 8 min with most of the diesel backflushed off the column, avoiding the detector. Diesel and similar heavy petrochemical streams often contain unwanted high molecular weight material that not only gets pushed backwards during the backflush phase, but whose removal is also aided by the PTV's higher temperature cleaning phase to ensure optimum cleanliness of the system.

Pre-column configuration

When set up in the pre-column configuration, a guard column is installed from the inlet to the microfluidic device, and the analytical column is connected from the microfluidic device to the detector. An uncoated, deactivated pre-column of 5 m x 0.32 mm, (P/N 26050-0532), is recommended. In this configuration, the backflush is turned on after the last analyte has entered the analytical column while the high boiling matrix stays behind in the pre-column. The reversed carrier flow in the precolumn backflushes these heavier compounds through the split line, while the analytes continue to flow forwards through the analytical column to the detector. This configuration has the advantage of protecting the analytical column as well as the detector from the high boiling matrix and removing the high boiling compounds more quickly at a lower oven temperature during the backflush stage. Figure 6 shows stacked overlaid total ion chromatograms for a mixed pesticide standard in an agricultural matrix, acquired using a Thermo Scientific™ TSQ™ 9000 GC-MS/MS system with Advanced EI (AEI) ion source, run in timed-SRM mode, using a 30 m x 0.25 mm i.d. x 0.25 µm Thermo Scientific™ TraceGOLD™ TG-5SilMS analytical column (P/N 26096-1420), with backflush turned on after 5 min and after 8 min.

To further illustrate this process, consider the last eluting pesticide in this standard, which is chlorantraniliprole. If the backflush is switched on too early (5 min), this compound is not detected, (Figure 7A). If a longer time is used before the backflush is turned on (8 min), this compound is detected (Figure 7B) and any higher boiling matrix still in the pre-column will be backflushed from the system. Not only does the backflush completely prevent the heavy compounds from reaching the detector, but there is zero detectable mass even in the most sensitive, AEI-triple-quadrupole configuration.

The backflush technique is rapidly being adopted in agricultural pesticide screening methods, since the adoption of faster, simpler, and more generic methods of extraction like QuEChERS, with minimal dSPE clean-up, to facilitate higher sample throughput. Despite the obvious benefits, this sample preparation approach can result in extracts with high levels of matrix co-extractives, which inevitably lead to faster contamination of the GC-MS system. Hence, an efficient backflush is helpful to prevent contamination and extend system uptime with these challenging samples.

Additionally, an orange matrix was spiked with a pesticide mix and tested by using n=100 repeated injections with the backflush enabled and a similar number of injections with the backflush disabled. The inlet liner (Thermo Scientific™ LinerGOLD™, six baffled, P/N 453T2845-UI), septum, and chromatographic capillary column (TraceGOLD TG-5SilMS, P/N 26096-1420) were

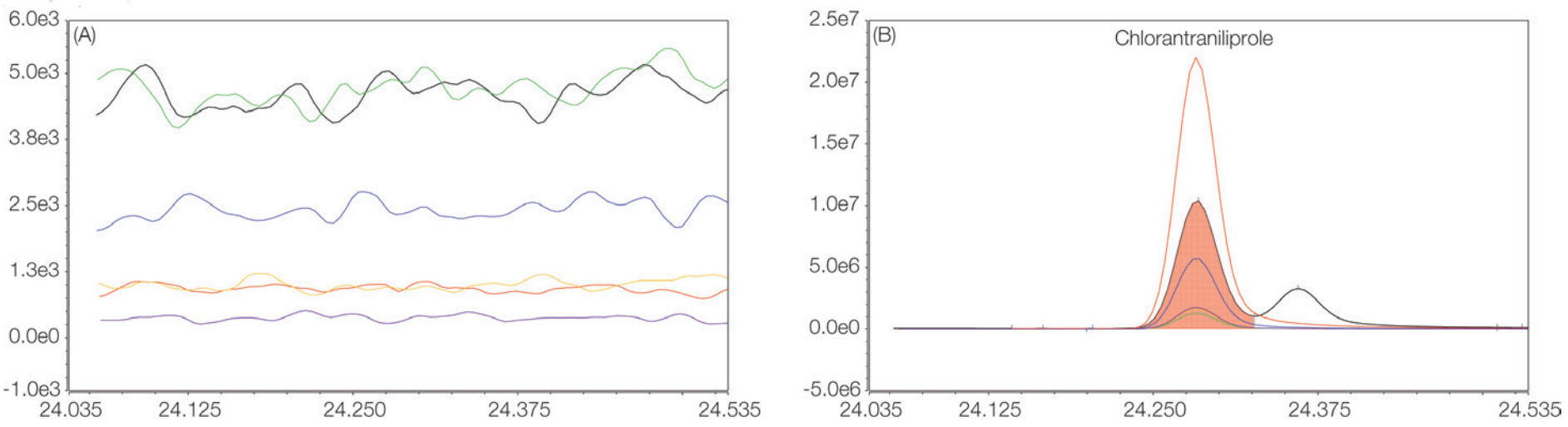


Figure 7. Extracted SRM chromatograms for the quantitation and five confirmation transitions for chlorantraniliprole showing no peak detected when an early backflush time is set, compared to a longer backflush time that allows for the elution of chlorantraniliprole (A) and a peak for chlorantraniliprole when a backflush time of 8 min is used (B), proving the effectiveness of the backflush system even for sensitive triple-quad applications

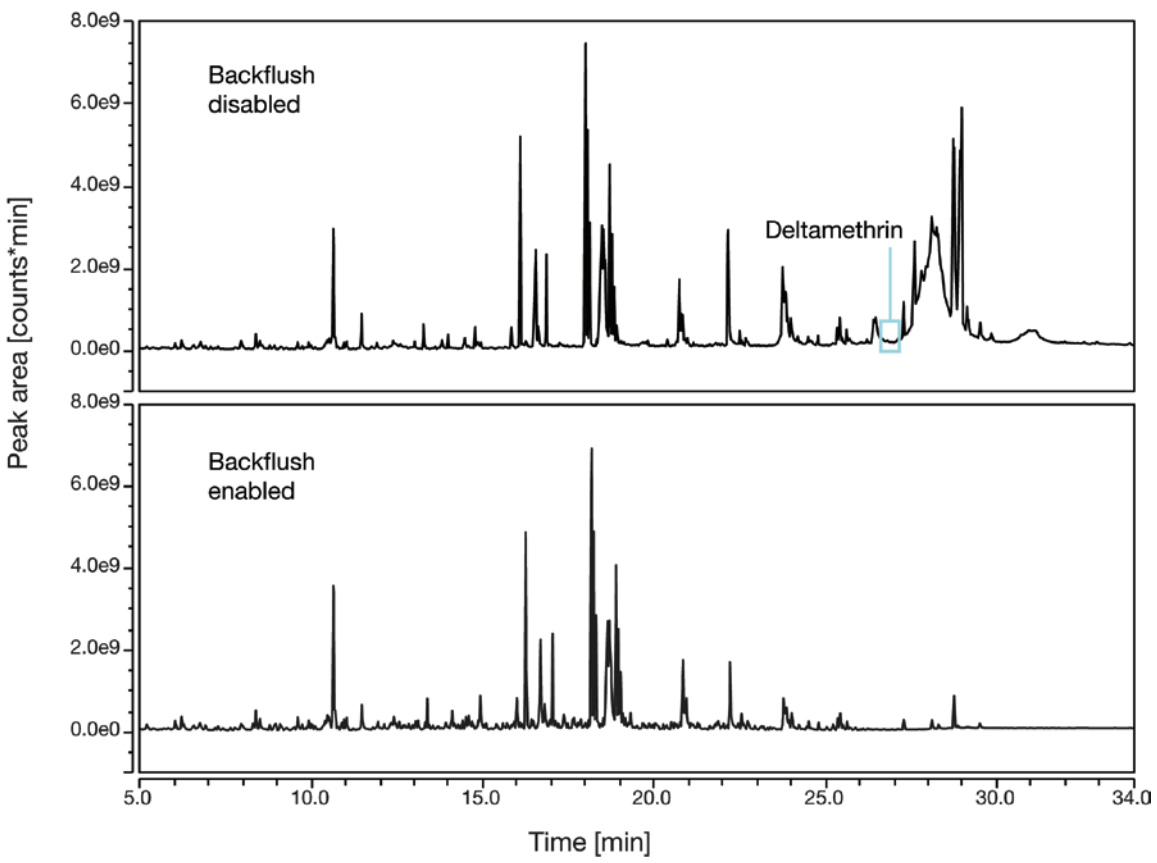
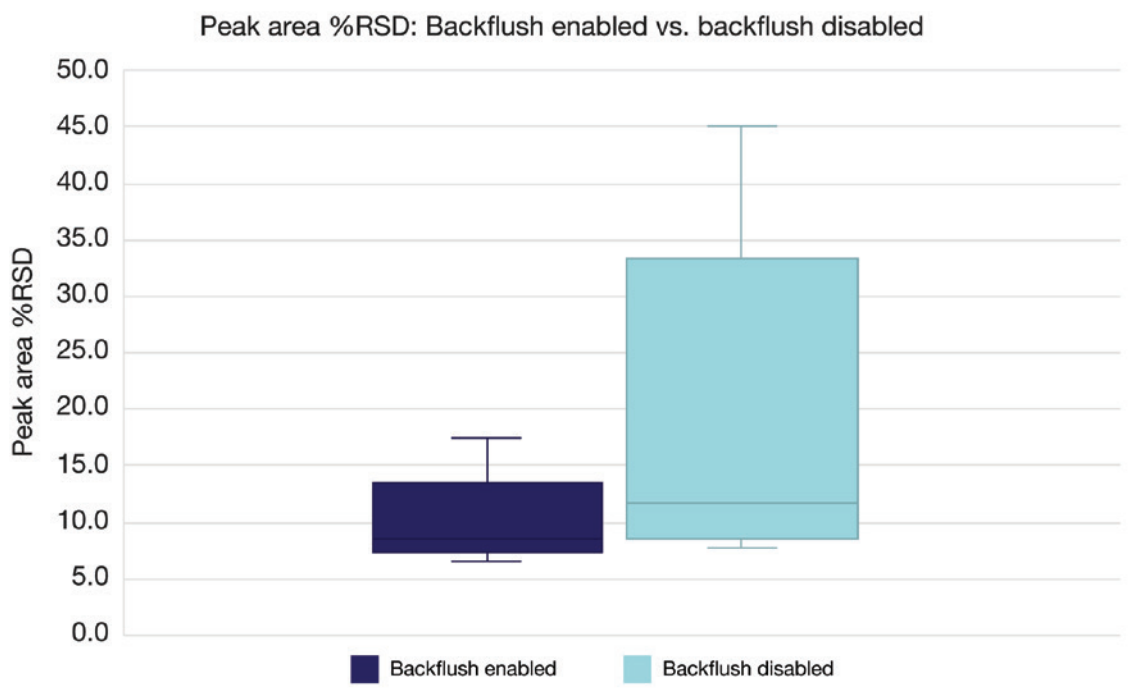


Figure 8. Orange is a complex matrix containing sugars, oils, terpenoids, and acids that elute along the chromatogram. The backflush option allowed the removal of the high boiler compounds eluting after the last peak of interest (deltamethrin, RT=26.70 minutes).



	Backflush enabled	Backflush disabled
Mean RSD%	9.1	16.6
P-value	6.083E-06	

Figure 9. Peak area %RSDs distribution for 40 pesticides over n=100 repeated injections of spiked orange matrix with backflush enabled and n=100 repeated injection with backflush disabled. The calculated p-value was <0.05, showing a statistically significant difference in the results.

changed between the two sequences. Ion source cleaning and mass spectrometer tuning (Thermo Scientific™ ISQ™ 7000 GC-MS equipped with a Thermo Scientific™ ExtractaBrite™ ion source) were performed for each experiment. Orange is a complex matrix containing sugars, oils, terpenoids, and acids (mainly citric acid) that elute along the chromatogram, affecting both the chromatographic performance and interfering with the target analytes (pesticides), thereby leading to loss of quantitative precision (Figure 8). The peak areas of targeted pesticides obtained from the two experiments were compared using a t-test, and the p-value (probability indicating significance level) was calculated. The p-value was <0.05, therefore representing a statistically significant difference between the results as reported in Figure 9. As an example, EIC for a mid-eluting compound (triallate) at different stages of the two sequences are reported together with the corresponding retention time deviation (relative to the initial retention time in the first injection) and peak asymmetry factor annotated (Figure 10). The comparison shows not only an improved RSD% and RT deviation when backflush is used, but a consistent response is maintained even after a hundred injections.

Assessment of analytical performance, PTV-backflush-GC-FID

One question that many people have before switching to backflush technology is whether performance parameters, such as retention time stability, repeatability of response, linearity, accuracy, and precision, will be affected as compared with non-backflush techniques. These parameters were tested using the post-column backflush configuration to ensure the integrity of results.

Retention time and peak area stability

Retention time and peak area stability were assessed by performing n=40 injections of a mixed alkanes standard using the iConnect PTV-Backflush and calculating the standard deviation of the retention time (SD RT) and the %RSD of the peak area. Table 1 shows the results for six compounds at 1 ppm in hexane using the iConnect FID for detection, indicating the iConnect PTV-Backflush remains consistent over longer injection sequences.

Linearity

To assess linearity, precision, and accuracy when PTV-backflush is enabled, a sample containing 50,000 ppm v/v diesel was spiked with cyclohexane, toluene, and p-xylene across the range 0–95 ppm v/v. Precision and accuracy were assessed in a sample spiked at 50 ppm v/v. The results of these tests with the backflush enabled at 8 min, are described below.

To obtain accurate quantification, a calibration curve is essential. Linearity was assessed by performing injections in duplicate across the range 0–95 ppm v/v for each of the three analytes. Excellent linearity was achieved across the range with R^2 values >0.999 and average calibration factor (AvCF) %RSDs <3.

Accuracy and precision

Precision and accuracy were assessed using n=20 injections of a 50 ppm v/v standard. An overlay of the chromatograms obtained is shown in Figure 12 along with the precision and accuracy values. The consistency of the results shows that there is no contribution from carryover of heavy diesel compounds, indicating that these compounds have been completely removed from the system by backflushing.

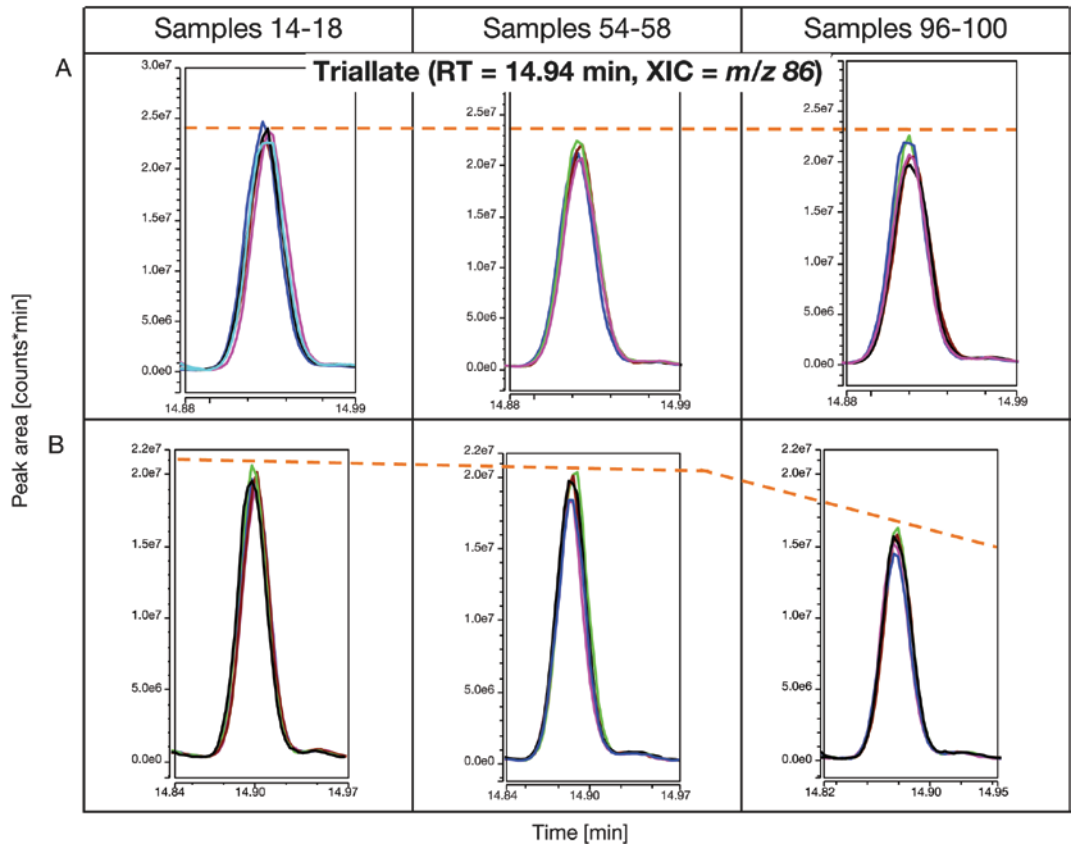
Summary

Backflushing is a valuable technique to prevent high boiling matrix compounds entering the analytical column and the detector, ensuring a more robust and reliable method, while reducing the run time, especially in the pre-column backflush configuration. This has several advantages over non-backflush methods. Utilizing backflush technology can help analytical testing laboratories dealing with difficult sample matrix to extend instrument uptime by reducing the required frequency of system maintenance. This can increase laboratory throughput and decrease running costs, all of which increase the laboratory's return on investment.

In summary, the iConnect PTV-backflush module offers:

- Integrated pneumatic control for an easy and self-adjusted pressure during backflush without the need for an additional auxiliary gas channel.
- The modular design of the TRACE 1600 Series GC offers the capability for the user to install the iConnect PTV-backflush module quickly and easily, at any time, without the need for a field service engineer.
- The use of the microfluidic connector based on SilFlow technology allows for simplified operation in either a pre-, post-, or mid- column configuration depending on the analysis needs.
- The pre-column backflush configuration assures the highest degree of robustness and sample throughput, preventing higher boiling matrix from entering the analytical column, greatly reducing the backflush time and therefore the overall analysis time.
- In the case of matrix compounds covering a wide volatility range, the post-column backflush configuration facilitates the setting of the backflush timing based on the separation achieved by the analytical column. This configuration effectively backflushes the matrix compounds from the analytical column, preventing possible detector contamination, reducing maintenance, and extending instrument uptime.

This technical note has been edited due to space limitations. For the full technical note on TRACE 1600 PTV Backflush, please visit thermofisher.com/gc



Average RT deviation (min, n=100)		Average tailing factor (As, n=100)		Absolute peak area %RSD (n=100)	
Backflush enabled	Backflush disabled	Backflush enabled	Backflush disabled	Backflush enabled	Backflush disabled
0.005	0.009	1.04	1.03	8.2	9.3

Figure 10. Examples of chromatogram traces for a mid-eluting compound (triallate) at different stages of the two sequences (A=backflush enabled, B=backflush disabled). Retention time deviation and asymmetry factors across the two sequences (n=100 injections each) are reported in the table. Without backflush a detrimental effect on the response, RSD%, and RT deviation is observed.

Table 1. Standard deviation of retention time and %RSDs of peak area shown for a mixed alkanes standard (n=40 injections) using the PTV-Backflush module in the post-column

	PTV-backflush (n=40)	
	SD RT (min)	%RSD area
Decane	0.0056	1.27
Dodecane	0.0048	1.14
Tetradecane	0.0047	1.11
Hexadecane	0.0047	1.13
Octadecane	0.0049	1.13
Eicosane	0.0052	1.12

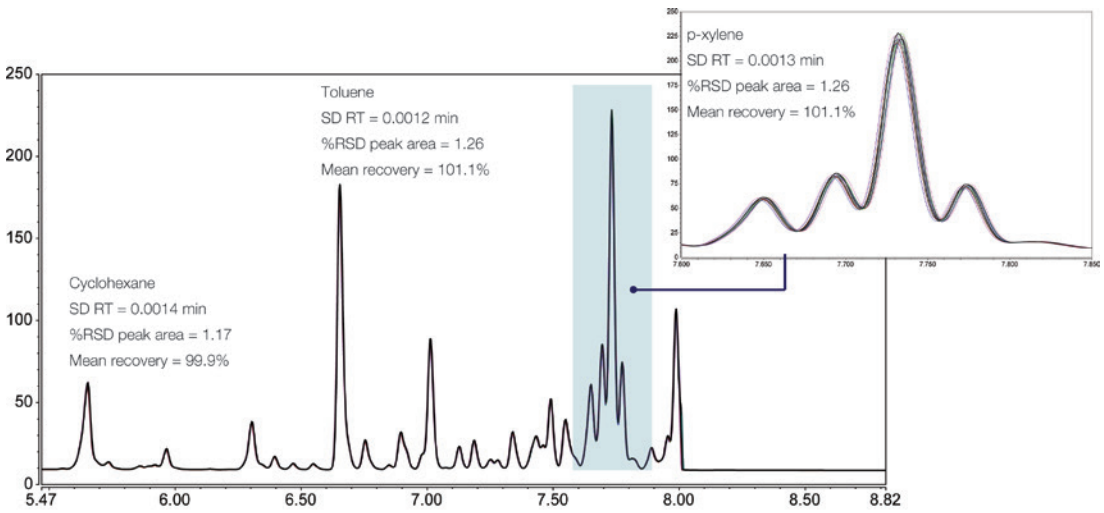


Figure 12. Overlaid FID chromatograms showing n=20 repeat PTV-backflush injections of a 50 ppm v/v standard with backflush enabled at 8 min. Target peaks are annotated with standard deviation of retention time (SD RT), %RSD peak area, and mean recovery. The inset shows a zoomed in area of p-xylene.

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