Capillary Flow Technology for GC/MS: Efficacy of the Simple Tee Configuration for Robust Analysis Using Rapid Backflushing for Matrix Elimination

Application

Environmental

Authors

Harry Prest
Agilent Technologies, Inc.
5301 Stevens Creek Blvd.
Santa Clara, CA 95051
USA

Christian Foucault and Yves Aubut
Agilent Technologies Canada, Inc.
2250 boul Alfred Nobel
St-Laurent, Québec
PQ H4S 2C9 Canada

Abstract

A previous application [1] described a simple Capillary Flow Technology (CFT) arrangement for GC/MS that provides minimal loss in MS signal, rapid backflushing, and quick servicing of the injection port and the head of the GC column without MS venting and operation in constant flow with pulsed injections. This arrangement uses a "tee" or purged union at the midpoint of two capillary GC columns with makeup flow controlled by electronic pressure control (EPC) devices. This application illustrates the improvement in run-to-run response robustness in a biological sample using the pressure-controlled tee (PCT) arrangement.

Introduction

Capillary Flow Technology (CFT) devices offer many opportunities for improvements in analytical quality. From the point of view of GC/MS, one major improvement is the capability of removing the late-eluting or “high-boiling” components that appear in the chromatogram after the last analytes of interest. Typically these are “removed” by increasing the oven temperature and adding additional run time to “boil” these off the column. However, this widely applied practice sends these contaminants off the column and into the MSD ion source. The net outcome is to reduce analyte response due to fouling of the MSD ion source and add analytical time. Ultimately, the result is lowered sample throughput due to long oven cycle times, extensive downtime required for ion source cleaning, and lowered run-to-run analytical quality because of the decreasing compound responses over time. Using the pressure-controlled tee (PCT) arrangement, the previous application [1] demonstrated how to rapidly eliminate these late eluters and shorten run times without substantial loss in signal. This application demonstrates how the PCT can improve the robustness of run-to-run analyte response using a biological sample acquired in positive chemical ionization (PCI) mode as a working example. This is significant for several reasons. First, chemical ionization modes are selective and so “blind” to many contaminants that can be detrimental to the analysis, thus eliminating them is a valuable advance. Secondly, in terms of robustness, the ion source trend is roughly:

Electron Impact Ionization ≥ Positive Chemical Ionization > Electron Capture Negative Ion Chemical Ionization (ECNICI)

Demonstrating enhanced robustness in PCI mode

will indicate the ability to protect the source in the other modes.

Experimental

Figure 1 shows a schematic of the instrument configuration for this analysis. The prior method utilized a single, continuous 50-m column and comparative data was acquired in this typical configuration. For the PCT configuration, two 25-m columns were used, with one ahead and one behind the tee, with the rear injection port controlling the tee flow as in the reference 1. The CFT tee was the Purged Ultimate Union (G3186-60580). All connections to the tee were made with the appropriate ferrules and fittings; most importantly, the MSD transfer-line connection was made with SilTite fittings.

The samples for this example were blood samples prepared for analysis of the lipid peroxidation product, 4-hydroxy-2,3-nonenal (HNE), which is considered an indicator of oxidative stress, and its metabolite, 1,4-dihydroxynonene (DHN). The preparation is extensive, [2] with addition of preservatives and reductive agents, steps for lipid removal, etc., but the resulting sample is still complex. Selective detection utilizing PCI with ammonia was chosen to simplify the detection and improve the quantitative determination. The MSD ion source was operated at 300 °C and the quadrupole at 150 °C and indicative ions were chosen for selected-ion monitoring analysis [2].

Results and Discussion

A reconstructed total ion current (RTIC) chromatogram acquired in full-scan mode using a single continuous 50-m column (without PCT), Figure 2, shows that even with the selective PCI and with the most “gentle” CI reagent gas, the sample still is very complex and the analytes are diminutive compared to the other matrix components. Especially intense are the late-eluting biologicals, which are known to “foul” the column phase; removing these required the oven program to extend to 340 °C and remain there for 3 minutes. This process improved the chromatographic performance by restoring the column phase; but driving these components into the ion source

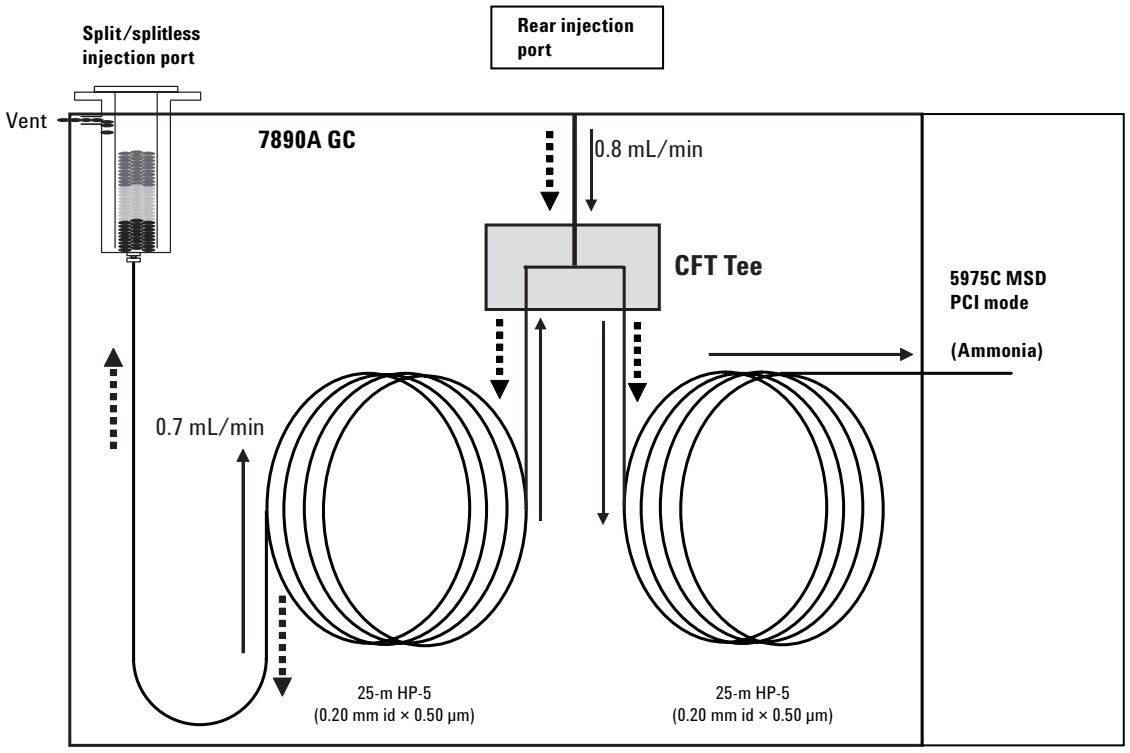

Figure 1. Schematic of pressure-controlled tee configuration for this analysis.

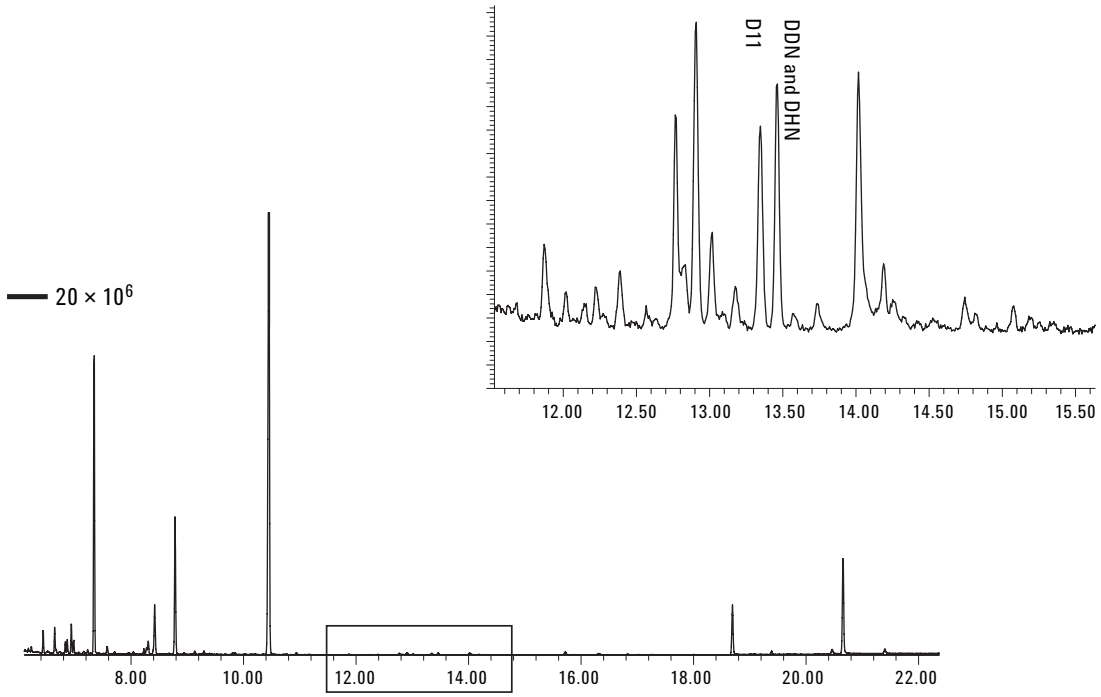

Figure 2. RTIC chromatogram of PCI-NH_3 full-scan acquisition of a typical sample. Note the intense, late-eluting (> 14 min.) components.

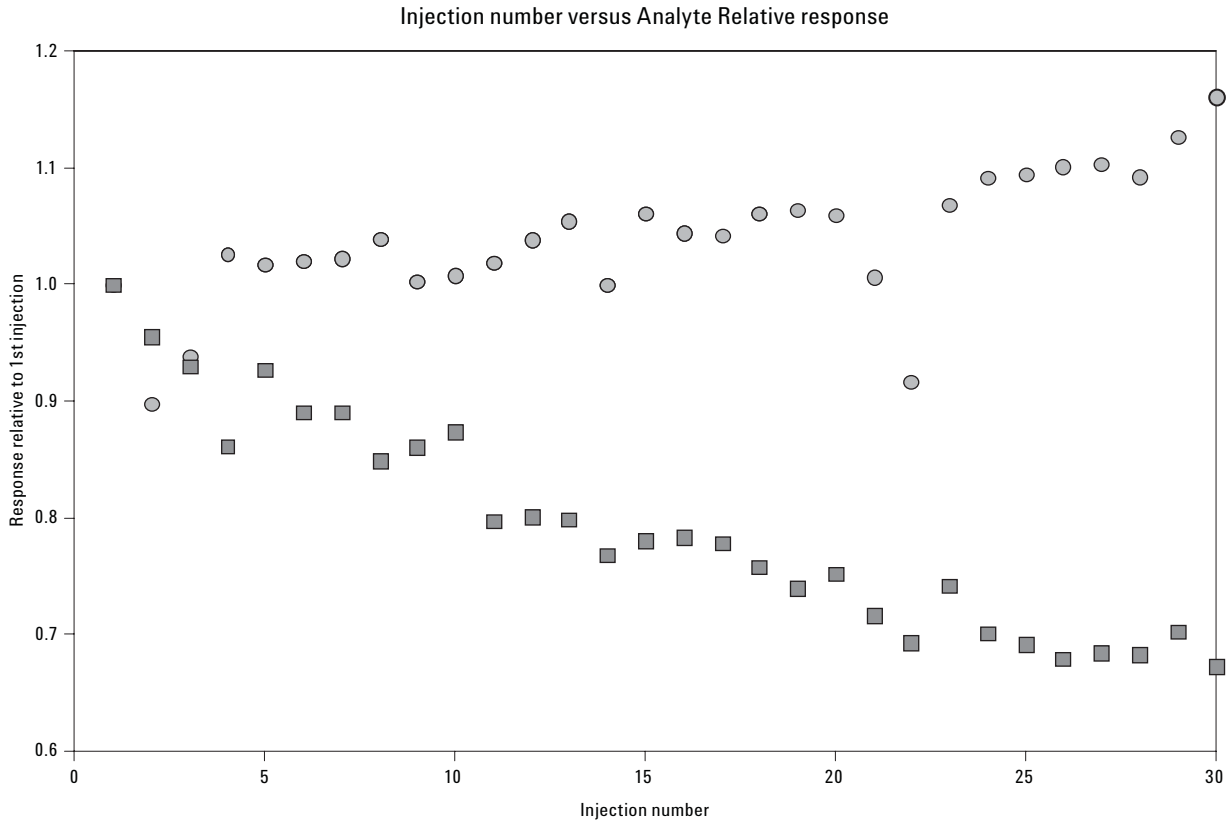

Figure 3. Analyte response versus injection number using the PCT with backflushing (circles) and using a continuous column without backflushing (squares).

rapidly degraded the analyte response. However, using the PCT configuration, these components were removed by backflushing them to the injection port and out the split vent. This improvement is shown in Figure 3. Using a continuous 50-m column configuration (without PCT or backflush), the analyte signal continuously drops and by the thirtieth injection more than 30 percent of the original intensity has been lost. Using the PCT and employing backflushing maintains signal and remains within about 10 percent of the first injection's response. (Some improvement in the PCI signal is seen as the clean source conditions in the course of the injections).

Conclusions

Using backflushing to remove late-eluting, matrix-related components can provide better uniformity in analyte response. This is especially important at trace concentrations, and it is at trace concentrations that the PCT configuration shows less signal loss than other CFT arrangements. Further, the ion source is more susceptible in PCI mode than EI so the improvement is more rapidly revealed. In ECNICI mode it may only take a few injections to lose response and, in this mode, the selectivity is such that many late eluters are invisible. The PCT is expected to be even more valuable in this mode.

Other advantages were found in reduced runtime and improved cycle time. Even with a rather conservative (that is, excessive) backflushing time and temperature (of 4 minutes), run time was shortened by 3.5 minutes and run-to-run cycle times can still be further optimized. The net result is higher sample throughput with higher quality data.

References

1. H. Prest, "Capillary Flow Technology for GC/MS: A Simple Tee Configuration for Analysis at Trace Concentrations with Rapid Back-Flushing for Matrix Elimination," Agilent Technologies publication 5989-8664EN
2. M. Veronneau, B. Comte, and C. Des Rosiers, "Quantitative Gas Chromatographic-Mass Spectrometric Assay of 4-Hydroxynonenal Bound to Thiol Proteins in Ischemic/Reperfused Rat Hearts," *Free Radical Biology & Medicine*, 33(10), p 1380–1388, 2002

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.

Printed in the USA
August 4, 2008
5989-9359EN

Agilent Technologies