Maintaining Compound Retention Times with the Backflush Enabled Pressure Controlled Tee Configuration for Agilent 7890A GCs with Agilent 5975 Series MSD and Agilent 7000 Series Triple Quadrupole MS Systems

Application Note

Environmental

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Abstract

The Pressure Controlled Tee configuration provides a simple approach to rapid back-flushing and GC-serving on all MS systems. To further improve acquisition and analy-sis, this note demonstrates how to generate compound retention times that are per-manent and universal through Retention Time Locking. If retention times can be per-manent on any system, then complex MS acquisition methods such as selected ion monitoring and multiple reaction monitoring modes need not be changed when columns are trimmed or replaced. Similarly, compound retention times are unchanged in data analysis which simplifies identification, quantitation and the use of databases of compound retention times. This note applies Retention Time Locking and the pres-sure Controlled Tee to analysis of the Japan Positive (Pesticide) List of about 430 compounds to add the feature of rapid backflushing without additional analytical time over the published method. The results show retention times may be reproduced from the cited database values to better than an average of 1-sec, which is a very good match. Rapid servicing of the column and inlet are possible without venting the MS system and applying simple "mechanical Retention Time Locking" quickly returns the GC/MS to acquisition without method changes or additional complexity.

Agilent Technologies

Introduction

Agilent's GC Capillary Flow Technologies (CFTs) have greatly expanded the capability and flexibility of gas chromatographic analysis. CFT can provide rapid column and inlet maintenance without MS venting as well as backflushing of the accumulated sample matrix preventing carryover, which compromises the column performance. One highly flexible CFT arrangement, the Pressure Controlled Tee (PCT) [1], has been shown to provide rapid backflushing and enhance analytical robustness in GC/MSD single quadrupole analysis in a very challenging sample matrix [2]. Other work has shown that introducing the PCT configuration maintains signal and therefore compound detection limits [3].

Another concern of analysts involves method maintenance, both in acquisition and data processing. After GC column maintenance or replacement, compound retention times change. This affects scheduled events in MS acquisition such as selected ion monitoring (SIM) or multiple reaction monitoring (MRM) modes as well as compound retention times in quantitation or library databases. One powerful approach to maintaining compound retention times is Retention Time Locking (RTL). Retention Time Locking provides a very simple idea: RTL makes compound retention times permanent and universal. This can greatly simplify methods and their development. An example is the Retention Time Locked compound database for the Japanese Positive List for pesticide analysis [4]. In this case, the retention times for 430 pesticides are "locked" to a particular column and oven program. Using this method of given compound retention times as an example, this note explores and addresses tools provided by the PCT for maintaining compound retention times in quantitation databases as well as for the SIM and selected reaction acquisition modes used by the Agilent 5973, 5975 and 7000 series Mass Spectrometers.

Experimental

Hardware and method parameters

Schematically the experimental arrangement is shown in Figure 1. The commonly used 30-m analytical column configuration is replaced by two 15-m columns joined by the Purged Ultimate Union. For these experiments, two 15-m DB-5ms (0.25-mm id × 0.25-µm film) were used (122-5512 UI). Makeup gas can be supplied by either an Auxillary Electronic Pneumatic Control (Aux EPC) or Pressure Control module (PCM) but here an Aux EPC was used. The Column 2 flow is always set slightly higher than the Column 1 flow by 0.04 mL/min to prevent back-diffusion in the makeup gas line. More details and the installation and use of the PCT are

described in the Rapid Universal GC/MS Backflushing Kit (G1472A) and included documents (Manual G1472-90001). An Agilent 5975 Series MSD was used but results are independent of the MS system and so apply to the 7000 Series Triple Quadrupole.

Details of the Japan Positive List method are provided [4] and only those altered to accommodate the PCT and backflushing are cited here (Table 1). Note the original method has an extended oven hold at 300 °C. This has been shortened by 5 min, and 3 min of that time has been incorporated for backflushing – overall the method is slightly shorter.

Table 1.   GC Parameters for RTL with Backflush

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Configuration:	(the actual arrangement is two 15-m columns but the GC is configured as follows)
Column 1:	Front Inlet to MSD Outlet: DB-5ms 30-m × 0.25-mm id × 0.25 µm film
Column 2:	AUX EPC Channel 4 to MSD Outlet: DB-5ms 15-m × 0.25-mm id × 0.25 µm film.
Carrier and mode:	Helium in constant flow mode
Column 1 flow:	As required
Column 2 flow:	= Column 1 + 0.04 mL/min
GC oven:	50 °C for 1 min, 25 °C/min to 125 °C for 0 min, 10 °C/min to 300 °C for 5 min
Post run:	300 °C for 3 minutes
Column 1:	0.25-mL/min
Column 2:	3-mL/min (or as allowed by the pumping system)

Results

RTL Experiments

In order to lock the method, the helium carrier flow must be set to produce a retention time of 13.443 min for the compound chlorpyrifos-methyl. To do this, the column flows for both Column 1 and Column 2 are manually incremented by 0.1 mL/min and the elution of the chlorpyrifos-methyl "locking compound" is determined by injection of a standard at each flow setting. The data and resulting regression are given in Table 2 and Figure 2, respectively. Note that more flow situations were acquired than necessary and usually only 5 flows would be required. The regression correlation is very high and, using the equation for the regression, it was calculated that a Column 1 flow of 1.47 mL/min and Column 2 flow of 1.51 mL/min should produce a retention time of 13.443 min for chlorpyrifos-methyl. These values were applied to the GC acquisition method and several standards containing chlorpyrifos-methyl and other compounds were acquired. The results showed a high degree of agreement not only for the locking compound but also other compounds in the Japan Positive List screening database (Table 3). In general,
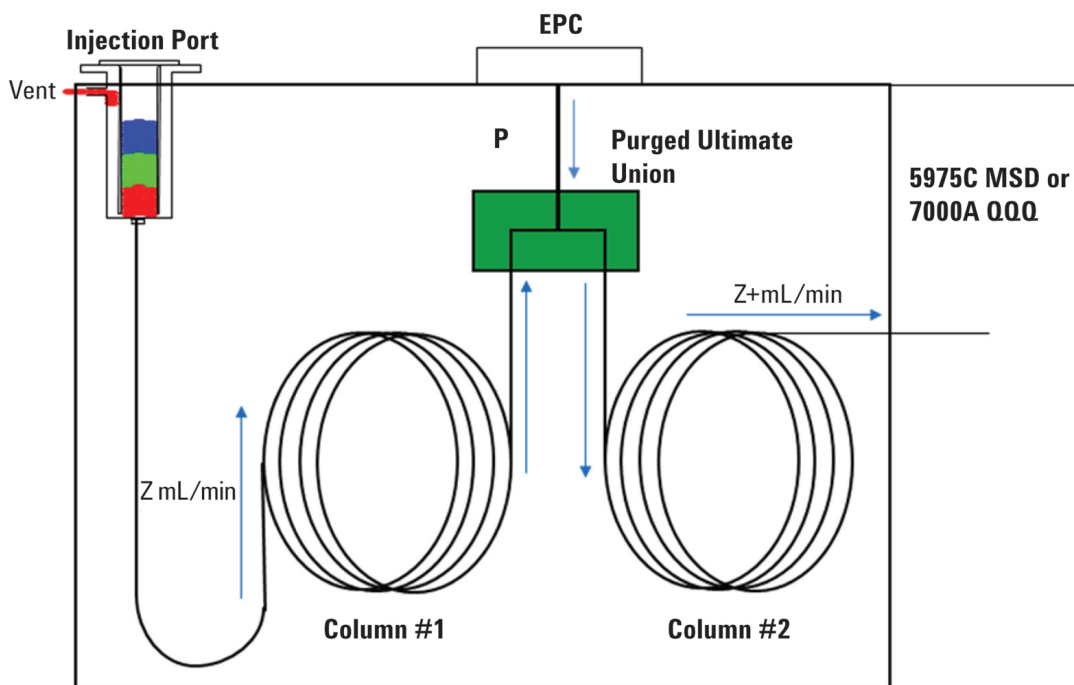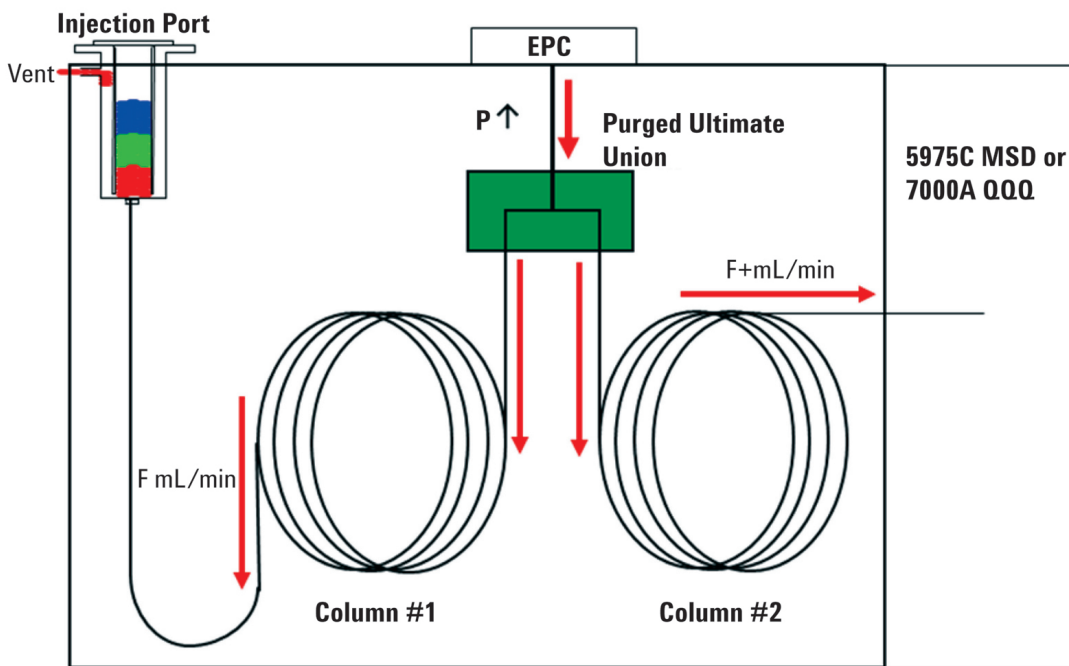
Figure 1.   *Schematic of the Pressure Controlled Tee configuration illustrating the arrangement. Upper panel shows the forward flow during analysis and the lower panel shows the column flows during backflushing.*

retention times agreed within better than 1 sec of their expected time. The worst case was on the order of 2 or 3 sec but considering the peak widths are on the order of 3 to 6 sec, this must still be considered within the analytical identification window. (The data also suggest a slight systematic error across the elution order which could be corrected to improve the fit on a permanent basis.)

Mechanical Retention Time Locking

Pesticide analysis typically involves intensive column and inlet maintenance. Setting the PCT in Backflush mode and cooling the injection port (and oven), allows the septum, liner and column to be rapidly serviced [5]. Usually a column "loop" of about a half-meter is cut off the column head. One approach to restoring retention times would be to check the new retention time of the locking compound via an injection and correct accordingly. However, Agilent Capillary Flow Technology (CFT) enables a more rapid and convenient approach. If the removed section of column is simply replaced with an equal length of capillary column, here DB-5ms 0.25-mm × 0.25-µm, then the retention times will remain unchanged. This is possible using the Ultimate Union as the column connector which is included in the G1472A kit. The Ultimate Union conveniently mounts inside the bracket next

Table 2.   Column 1 (30 m) and Column 2 (15 m) Flow Settings and the Measured Retention Time of the Chlorpyrifos-methyl Locking Compound.

Column 1 flow	Column 2 flow	Time (min)
1.00	1.04	14.164
1.10	1.14	13.978
1.20	1.24	13.814
1.30	1.34	13.665
1.40	1.44	13.529
1.50	1.54	13.406
1.60	1.64	13.292
1.70	1.74	13.185

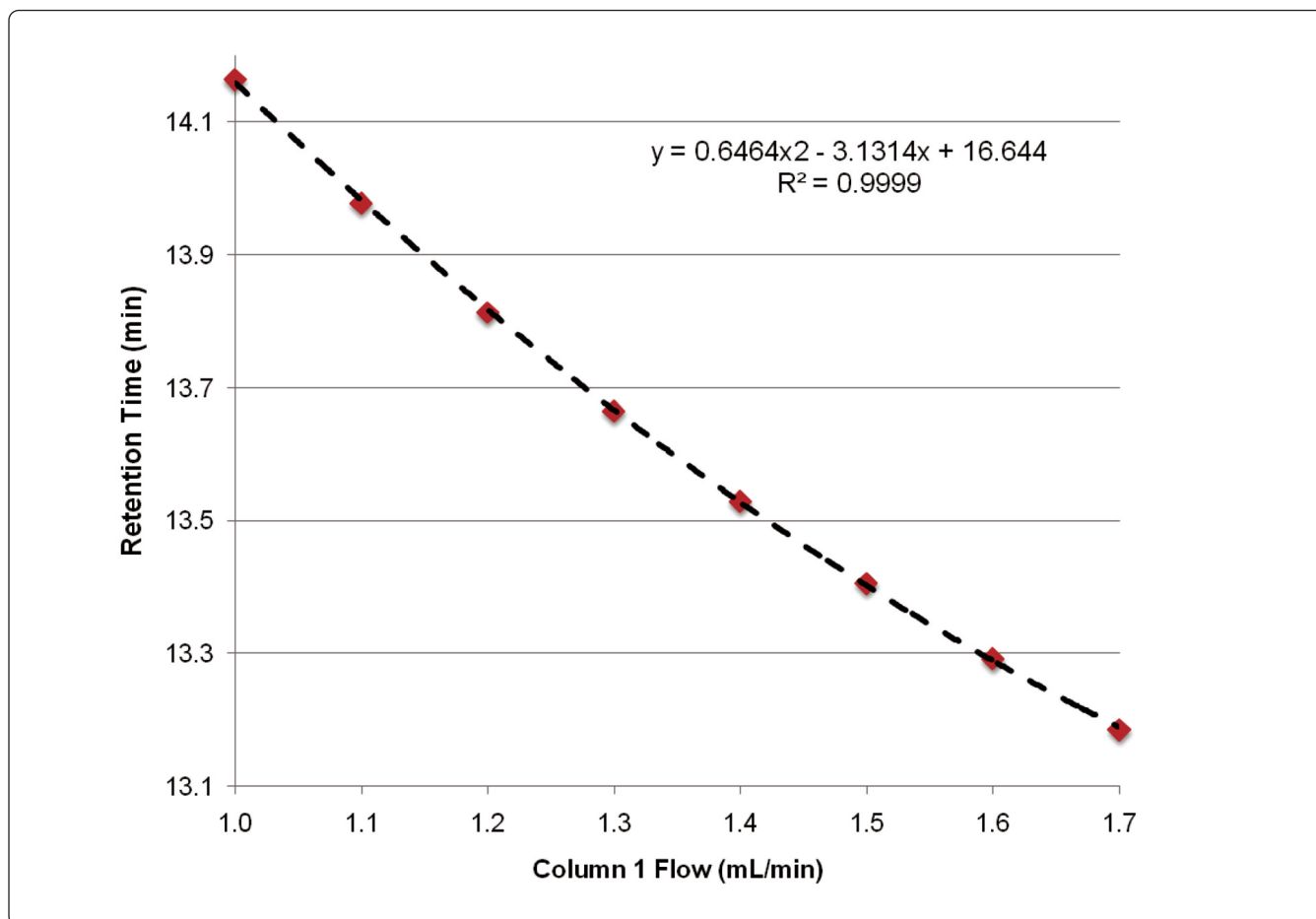$$y = 0.6464x^2 - 3.1314x + 16.644$$
$$R^2 = 0.9999$$

Figure 2.   Regression of locking compound retention time versus column 1 flow.

to the Purged Ultimate Union (Figure 3). Employing this approach, a column loop of the DB-5ms column at the injection port was removed and replaced with the Ultimate Union connection. Standards were rerun with no other change to the GC acquisition method and the results are shown in Table 4. Again, deviations from expected retention times are very small, less than 1 sec and much less than a peak width, suggesting MRM and SIM tables would not require amendment.
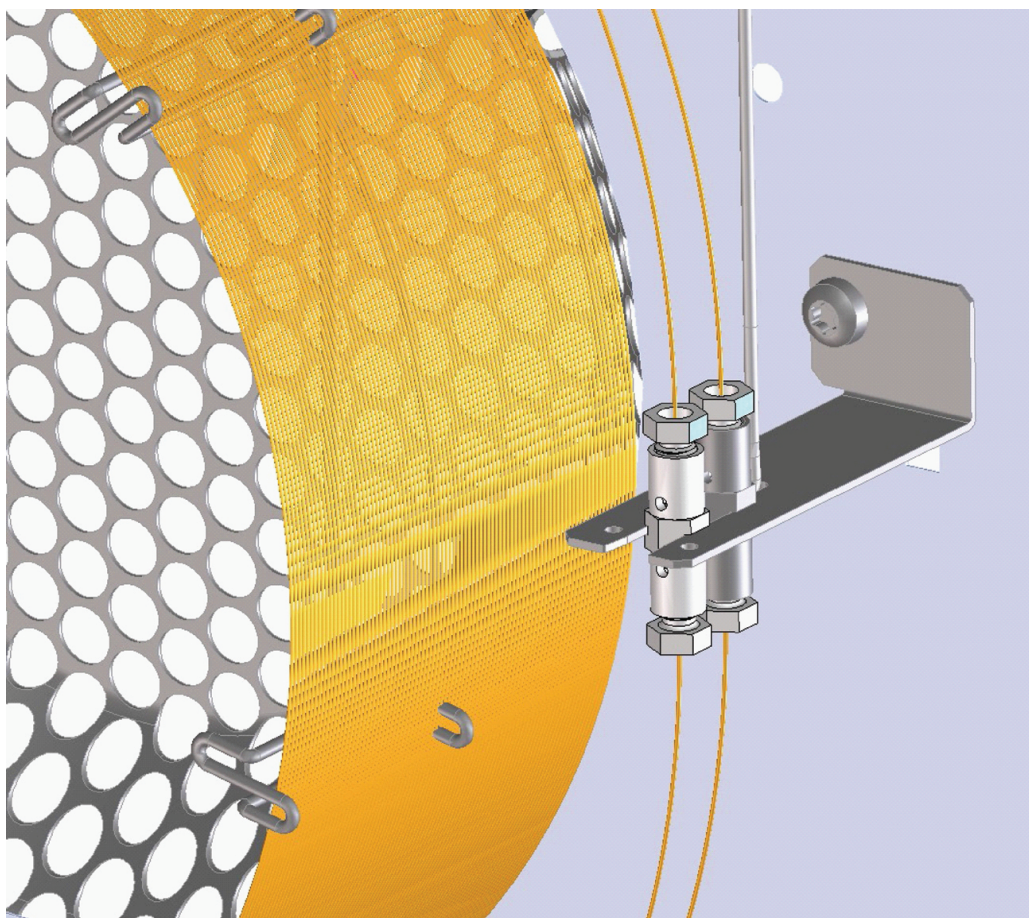
Figure 3.   Schematic illustration of the PCT configuration and mounting bracket: The Purged Ultimate Union is mounted behind the Ultimate Union. The Purged Ultimate Union is at the mid-point of the columns (gold lines) while the Ultimate Union connects a short pre-column to the injection port. Not shown is the retaining pin which secures the union.

Table 3. Agreement between Measured Compound Retention Times and the Expected Retention Times for Selected Compounds in the Japan Positive List Database. Columns A, B, and C Refer to Multiple Measurements for Compounds Present in Different Analyzed Standards.

Compound	RT (measured-expected) sec.		
	a	b	c
Dichlorvos	−0.66	−0.66	
Mevinphos	−0.66	−0.66	
Ethoprophos	−0.66	−0.9	
Naled	−0.48		
Sulfotep	−0.36		
Phorate	−0.36	−0.96	
BHC alpha isomer	−1.14	−1.02	
Lindane	−0.72		
Fonofos	−0.12		
Diazinon	0.00	−0.48	
Disulfoton	0.06	0	−0.72
BHC delta isomer	−0.72		
Chlorpyrifos Methyl	0.24		
Methyl parathion	−0.12	−0.78	
Heptachlor	−0.06		
Fenchlorphos	0.24	-0.42	
Fenitrothion	0.48		
Malathion	0.36		
Chlorpyrifos	0.24	0.24	−0.06
Fenthion	0.42	−0.36	
Aldrin	0.36		
4,4'-Dichlorobenzophenone	0.18		
Heptachlor exo-epoxide	0.6		
Tetrachlorvinphos	0.42	0.12	
Endosulfan (alpha isomer)	0.36		
Fenamiphos	1.08		
Prothiofos	0.6	0.06	
p,p'-DDE	0.3		
S,S,S-Tributylphosphorotr	1.02	0.48	−0.18
Dieldrin	1.02		
Endrin	0.24		
Fensulfothion	0.96	-0.06	
Ethion	1.08		
p,p'-DDD	0.84		
o,p'-DDT	−2.28		
Sulprofos	1.14	0.18	
Carbophenothion	1.02		
Endosulfan sulfate	1.5		
p,p'-DDT	0.84		
EPN	1.56		
Azinphos-methyl	1.02	0.36	
Azinphos-ethyl	1.86		
Coumaphos	2.7	2.34	

Table 4. Results for "Mechanical Retention Time Locking" by Use of the Ultimate Union Connector

Compound	RT Error (s)
Dichlorvos	−0.66
Mevinphos	−0.66
Ethoprophos	−0.9
Sulfotep	−0.36
Phorate	−0.96
Fonofos	−0.54
Diazinon	−0.48
Disulfoton	−0.48
Chlorpyrifos Methyl	0.06
Methyl parathion	−0.78
Fenchlorphos	−0.42
Fenitrothion	−0.42
Malathion	−0.12
Chlorpyrifos	−0.12
Fenthion	−0.36
Tetrachlorvinphos	0.12
Fenamiphos	0
Prothiofos	0.06
S,S,S-Tributylphosphoro	0.12
Ethion	0.48
Sulprofos	0.18
Carbophenothion	0.18
EPN	0.42
Azinphos-methyl	0.36
Azinphos-ethyl	0.72
Coumaphos	2.34

Conclusions

Maintaining compound retention times can greatly simplify acquisition and data analysis methods in GC/MS or GC/MS/MS. The PCT configuration can easily be used to generate permanent and universal retention times for compounds through Retention Time Locking. To show the "universal" attribute, it has been demonstrated that given the initial Japan Positive List method, RTL with the PCT can replicate the 430 compound retention times to a very high degree. By extension, any method and database can be replicated. Using a replaceable section of column which can be rapidly exchanged during servicing (without venting or cooling the MS), compound retention times can be conserved and can be considered permanent. This was demonstrated in constant carrier gas flow mode as this provides the best chromatographic conditions for GC/MS and is the normal approach of MS users. The Ultimate Union, like the Purged Ultimate Union, has very low dead volume, low activity, and minimal influence on chromatography. Overall, the Pressure Controlled Tee configuration provides a number of simple strategies for improving method robustness and method maintenance without loss in sensitivity.

References

1   "Capillary Flow Technology for GC/MS: A Simple Tee Configuration for Analysis at Trace Concentrations with Rapid Backflushing for Matrix Elimination," Agilent Technologies publication 5989-8664EN

2   "Capillary Flow Technology for GC/MS: Efficacy of the Simple Tee Configuration for Robust Analysis Using Rapid Backflushing for Matrix Elimination," Agilent Technologies publication 5989-9359EN

3   "Implementation of the Pressure Controlled Tee for Backflushing for the 7000 Series Triple Quadrupole Mass Spectrometer: Implications for Sensitivity," Agilent Technologies publication 5990-4504EN

4   Screening for Pesticides in Food Using the Japanese Positive List Pesticide Method: Benefits of Using GC/MS with Deconvolution Reporting Software and a Retention Time Locked Mass Spectral Database, 5989-7436EN

5   "The Rapid Universal GC/MS Backflushing Kit," part number G1472A and contains the manual (G1472-90001) which describes this configuration and operation for 5973 or 5975 or 7000 series Mass Selective Detectors.

Acknowledgement

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