The Application of Retention Time Locking in the Gas-Chromatographic Determination of Organophosphorus Pesticides in Lemons

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Introduction

Gas chromatography with mass spectrometric detection (GC-MS) has been widely applied for the analysis of pesticides in fruit for many years. Multi-component GC-MS determination, using on-column injection combined with selected ion monitoring (SIM), is routinely employed at the SASA laboratory. Although SIM is one of the most sensitive mass detection methods, it is also time consuming to set up, requiring careful adjustment of the appropriate data acquisition time windows. A major disadvantage in the routine application of this type of complex analysis, is the lack of reproducibility of retention times, caused by sample matrix effects and by column maintenance activities. Here we report on the use of retention time locking (RTL) as an aid to identification and quantitation of analytes in automated data processing operations, as applied to the GC-MS analysis of organophosphorus pesticides in lemons.

Experimental

Chromatographic Parameters:

Instrument	Agilent 6890 GC System	
GC column 1	DB5MS 26.5m x 0.25mm I.D. x 0.25µm	
GC column 2	DB5MS 30m x 0.25mm I.D. x 0.25µm	
GC column 3	Zebron ZB5 30m x 0.25mm I.D. x 0.25µm	
Retention Gap	Deactivated fused silica 1m x 0.53mm I.D.	
Carrier gas	Helium	Flow and Mode 1.2ml/min, constant flow
Injection Mode	Cool on column	Injection volume 1µl
Injector Temperature Program	50°C (0.1min) increasing @ 140°C/min to 250°C (15min)	
Oven Temperature Program	50°C (2min) increasing @ 25°C/min to 200°C, increasing @10°C/min to 240°C/min, finally increasing @40°C/min to 275° (4min)	

Mass Spectrometer Parameters:

Instrument	Agilent 5973N MSD	Quadrupole Temperature	150°C
Solvent Delay	9 min	Source Temperature	230°C
Scan Mode	Single Ion Monitoring (SIM)	MSD Transfer Line Temp	280°C
Data System	Chemstation Version C.00.01		

Results and Discussion

On-column injection procedures require frequent maintenance to the injection port and replacement of the retention gap/guard column to maintain optimum chromatographic performance. These actions introduce variability in the retention times of the pesticides, and although the changes are small, the SIM windows used for data acquisition need to be updated to ensure analyte detection and quantitation. Details of the initial SIM acquisition windows and individual retention times for all pesticides are listed in Table 1. Repeated use of the initial time windows, set up on Day A, would result in several pesticides not being detected on separate days. Pirimiphos-methyl, for example, was not detected on Days B or C because the retention times of 11.27 and 11.32 min. were out with the original SIM window (11.06 – 11.26 min.). Although adjustment of the SIM windows would address any changes in retention times, automated quantitation procedures are also adversely affected. Signal extraction time windows in the quantitation database have to be adjusted to ensure pesticide quantitation. Data illustrating the effect of variations in retention time on the automated identification process and the importance of assigning the appropriate signal time window are shown in Table 2.

Table 1. Initial acquisition windows and actual retention times following column maintenance (Column 2.)

		Pesticide Retention times (min)			
Pesticide	SIM window(min)	Day A	Day B	Day C	Day D
diazinon	10.10-10.41	10.16	10.20	10.24	10.18
chlorpyrifos-methyl	10.41-10.91	10.86	10.91	10.96	10.89
parathion-methyl	10.91-11.06	10.96	11.00	11.05	10.98
pirimiphos-methyl	11.06-11.26	11.22	11.27	11.32	11.24
fenitrothion	11.26-11.35	11.30	11.34	11.39	11.32
malathion	11.35-11.46	11.39	11.44	11.49	11.41
chlorpyrifos-ethyl	11.46-11.58	11.52	11.57	11.62	11.55
parathion-ethyl	11.58-11.72	11.65	11.70	11.74	11.67
pirimiphos-ethyl	11.72-11.98	11.80	11.85	11.90	11.83
chlorfenvinphos	11.98-12.33	12.17	12.23	12.26	12.20
methidathion	12.33-12.93	12.48	12.53	12.56	12.50
ethion	12.93-13.47	13.38	13.42	13.46	13.40
triazophos	13.47-14.06	13.55	13.60	13.64	13.58
phosmet	14.06-14.88	14.54	14.59	14.66	14.57
azinphos-methyl	14.88-15.50	15.21	15.28	15.35	15.25

Table 2. Influence of signal time windows on quantitation results

	Signal Time Window		
Quant. ion	±0.1 min	±0.15 min	±0.2 min
304	N.D.	0.51	0.51
286	N.D.	0.59	0.59
263	N.D.	0.20	0.20
290	N.D.	0.54	0.54
277	0.08	0.08	0.08
173	N.D.	0.41	0.41
314	N.D.	0.76	0.76
291	N.D.	0.20	0.20
318	N.D.	0.71	0.71
323	N.D.	0.38	0.38
145	0.54	0.54	0.54
231	N.D.	0.64	0.64
161	N.D.	0.23	0.23
160	N.D.	0.37	0.37
160	N.D.	N.D.	0.37

N.D. – not detected; results expressed in mg/kg

Setting up a retention time locked method involved the collection of data for a time lock reference compound (methidathion) at the standard column head pressure for the method, and at ±10% and ±20% of that standard pressure. These data were evaluated automatically and a pressure/retention time curve generated. Predicted column head pressures which would result in the time lock reference compound eluting at the desired time could be calculated and added to the method. Chromatographic data, for the mixture of pesticides acquired on 4 separate occasions following column maintenance is shown in Figure 1. The application of RTL is shown in Figure 2. The retention times for all 15 pesticides, following column maintenance on 3 separate occasions, gave excellent agreement and neither the SIM acquisition times nor the quantitation database required updating.

Figure 1. GC/MS chromatograms acquired using Column 2 on separate occasions after column maintenance.
Sample: mixture of pesticides in a lemon matrix (≡ 0.5-2.5ng on-column)

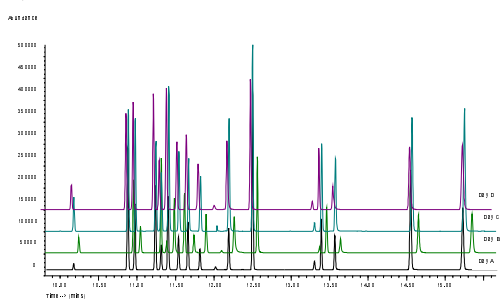
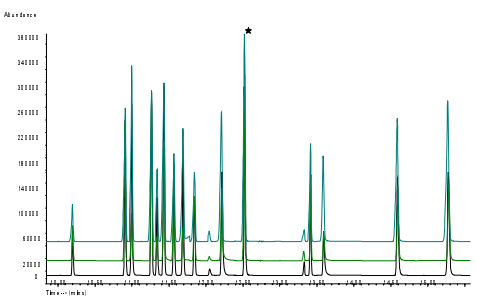


Figure 2. GC/MS chromatograms acquired using Column 2, on 3 separate occasions, using RTL method adjustment after column maintenance. Sample: As for Figure 1.
★ Locking compound



Despite the protection of a retention gap/guard column during routine operations, some sample matrix components migrate onto the analytical column, eventually degrading its performance to the point where replacement is necessary. Figure 3 shows the variation in retention times obtained from 2 columns of identical specification and source, but of differing length. Data obtained by applying retention time locking to this situation, is illustrated in Figure 4. The system was not able to adjust retention times to compensate for significant changes in column length, and some updating of acquisition windows was required to facilitate automated data processing.

Figure 3. GC/MS chromatograms acquired on different DB5-MS columns.
Sample: As for Figure 1.

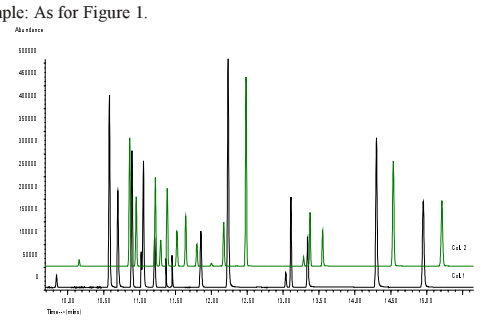
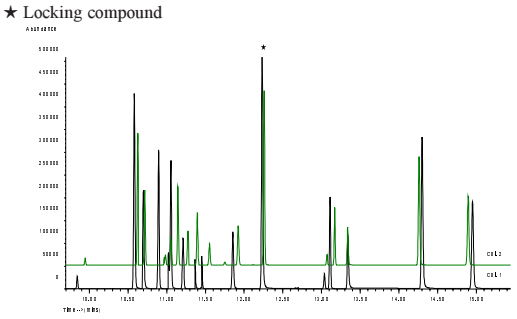


Figure 4. GC/MS chromatograms acquired on different DB5-MS columns using RTL method adjustment. Sample: As for Figure 1.
★ Locking compound



Variations in retention times obtained from 2 columns of the same specification and length, but supplied by different manufacturers, are shown in Figure 5. More general shifts in retention time were compounded by differences in resolution for some pairs of analytes. Again the application of RTL was not able to adjust the retention times to match the pre-set SIM windows (figure 6)

Figure 5. GC/MS chromatograms acquired on different manufacturers columns but of similar dimensions and phase.
Sample: As for Figure 1.

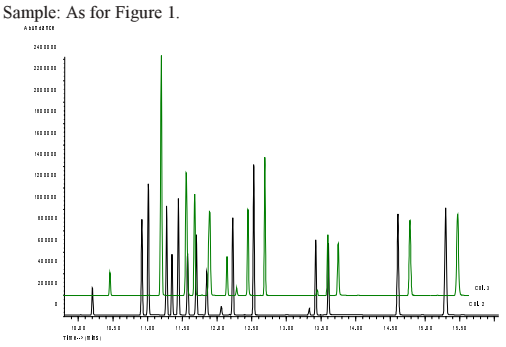
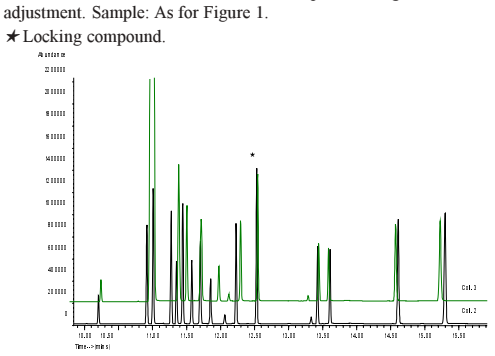


Figure 6. GC/MS chromatograms acquired on different manufacturers columns but of similar dimensions and phase using RTL method adjustment. Sample: As for Figure 1.
★ Locking compound.



Conclusions

The main advantage gained from the use of retention time locking was the ability to apply an existing data acquisition and processing method without amendment, after column/injector maintenance. In situations where retention gaps are replaced daily, savings in analyst time of up to 2.5 hours per week could be achieved. However in the examples discussed here, RTL did not accommodate more profound differences in retention times associated with significant variations in column length. Neither was it able to facilitate direct transfer of methods from column to column, of varying manufacturing source, without the modification of acquisition parameters.