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Addressing the challenges of changing retention times in GC/GC-MS

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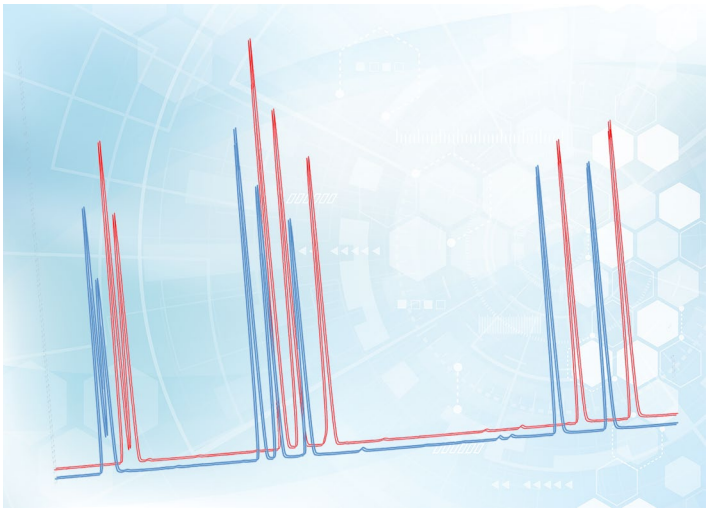

Goal

To demonstrate the applicability of the Thermo Scientific™ Retention Time Alignment (RTA) software for delivering consistent retention times for the same column configuration, irrespective of analytical column maintenance, GC or GC-MS system used.

Introduction

The chromatographic retention time is an important qualifying parameter often used to identify eluting compounds. The retention time of analytes can shift due to variation in sample matrices across the batch, instrument variations / drifts (pressures, temperatures, flows), as well as analytical column deterioration, trimming, or replacement.

When a column is replaced with another column with the same phase and dimensions, slight variation of the retention times is expected because actual column dimensions can vary from column to column. Also, trimming of the column during routine maintenance will result in shorter retention times. When multiple instruments are running the same method, consistent retention times are also critical. Any variations in compound retention time can lead to errors in peak identification, requiring additional time to check and correctly assign new retention times in the processing method.

In this work the RTA software was used to update the instrument method to maintain consistent retention times for compounds in different standard mixtures in chromatographic GC-MS analysis, after column maintenance. Examples using liquid and headspace injection techniques are demonstrated.

Experimental

Instrument and method setup

The RTA software was tested using two different GC-MS configurations:

1. Headspace GC-MS: A Thermo Scientific™ TriPlus™ 500 headspace (HS) autosampler was coupled to a Thermo Scientific™ TRACE™ 1310 gas chromatograph (GC) and a Thermo Scientific™ ISQ™ 7000 single quadrupole mass spectrometer. Chromatographic separation of a mixture of seven volatile organic compounds was achieved using a Thermo Scientific™ TraceGOLD™ TG-624 60 m × 0.25 mm × 1.4 µm column (P/N 26085-3330).

2. Liquid injection GC-MS: A Thermo Scientific TRACE 1310 GC was coupled to a Thermo Scientific™ Exactive™ GC Orbitrap™ HRAM-MS system and a Thermo Scientific™ TriPlus™ RSH autosampler. Chromatographic separation for a mixture of >200 pesticides was achieved using a Thermo Scientific™ TraceGOLD™ TG-5SilMS 30 m × 0.25 mm i.d. × 0.25 µm film capillary column with a 5 m integrated guard (P/N 26096-1425).

Retention Time Alignment software

The RTA software uses the retention time of a known reference compound (usually an alkane) during a single isothermal run to account for differences when column maintenance, column change, or method transfer has been performed. The reference retention time is established by running the GC isothermally, at the starting oven temperature of the analytical method, until the reference compound has eluted, and then ramping to a higher temperature to ensure all remaining compounds have eluted. All other method parameters remain unchanged.

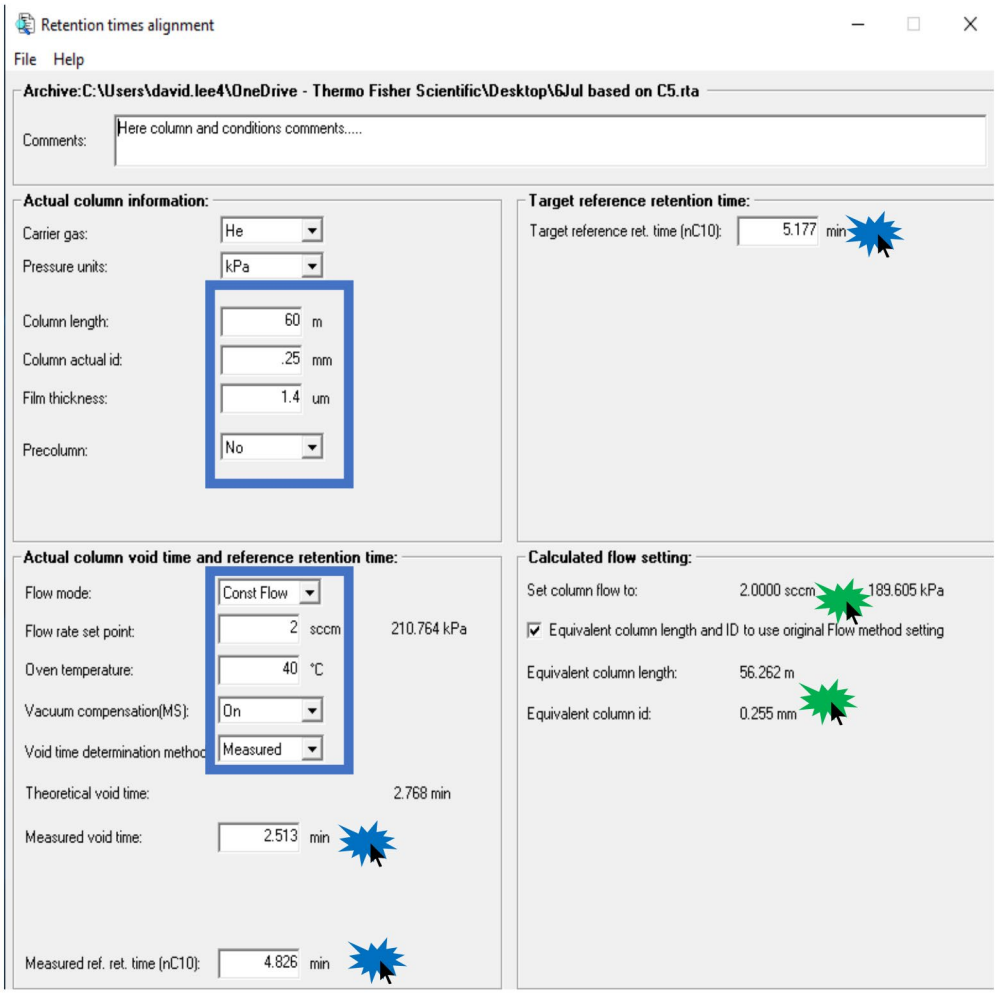

Figure 1. Screenshot of the Retention Time Alignment software (standalone desktop version), which calculates either the required column flow, maintaining column dimensions, or the required column length and internal diameter settings, while maintaining the column flow setting. Highlighted are the column parameters that need to be entered (blue boxes), the void time and retention times that need to be entered (blue stars), and the outcome as corrected resulting column flow/dimensions (green stars).

The RTA software is available as a standalone desktop version (Figure 1) or as part of Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software (version 7.2.8 or later). Highlighted in Figure 1 are the various column parameters that need to be entered and are used in the calculations, including the void time value, which can be taken from the GC user interface, the reference compound target and measured retention times.

Standards and sample preparation

For total vaporization HS injection, a mixture of seven VOCs was prepared at 25 µg/mL in methanol and 4 µL was added to a 20 mL headspace vial and sealed for analysis. For the reference compound, a 1000 µg/mL solution of pentane was prepared in isooctane and 20 µL was added to a 20 mL headspace vial and sealed for analysis. For liquid injection, a mixture of pesticides purchased from Restek (P/N 32562) was diluted to 5 µg/mL in acetonitrile. For the reference compound (nonane) a mixture containing C7-C30 alkanes was purchased from Sigma-Aldrich (P/N 49451-U) and diluted to 100 µg/mL with hexane.

Observations

Using the RTA software, adjustments can be made either to the column flow rate, set in the instrument method within the CDS, or to the column dimensions settings within the GC user interface (Figure 2). In both cases, the actual flow through the column will be altered; however, either the column dimensions or the flow rate settings will remain unchanged. Either method can be used depending on the requirements of the laboratory where the testing is being performed.

Figure 2. Picture of the column configuration settings window within the GC user interface

Initial retention times of the VOCs, pesticides, and reference compound retention times were established using their respective methods. Approximately 2 m was then cut from the inlet ends of the analytical columns and the reference compounds were analyzed again. The measured retention time of the reference compound was entered into the RTA software along with the target reference retention time, new void time, initial column dimensions, initial flow, and initial oven temperature. The column flow and column dimension settings required to maintain the retention time of compounds were then calculated by the software.

Retention time correction by altering column flow setting

After adjusting the column flow within the instrument method to those calculated by the RTA tool, the standard mixes were analyzed and the retention times compared to those in the original run. Figure 3 shows the RT differences with and without RTA adjustment. Setting the new calculated column flow resulted in retention time differences <0.02 minutes, well within the SANTE[1] retention time window guidelines of 0.10 min. An example of the retention time differences is illustrated in Figure 4 for three VOC compounds (D_2-dichloromethane, D_8-toluene and D_4-1,2-dichlorobenzene), which shows the extracted ion chromatogram (EIC) initially and after column trimming, both with and without retention time adjustment.

Retention time correction by altering column settings

After adjusting the column dimensions within the GC configuration panel (Figure 2), to those calculated by the standalone RTA software (note the Chromeleon CDS version of the software does not include this calculation), the standard mixes were analyzed and the retention times were compared to the original run. Results for selected compounds are shown in Figure 5. All the retention times are maintained within the 0.10 minute window allowed within the SANTE guidelines. Without RTA, the analytes would be outside of the identification retention time windows of the method so would be incorrectly assigned or missed altogether.

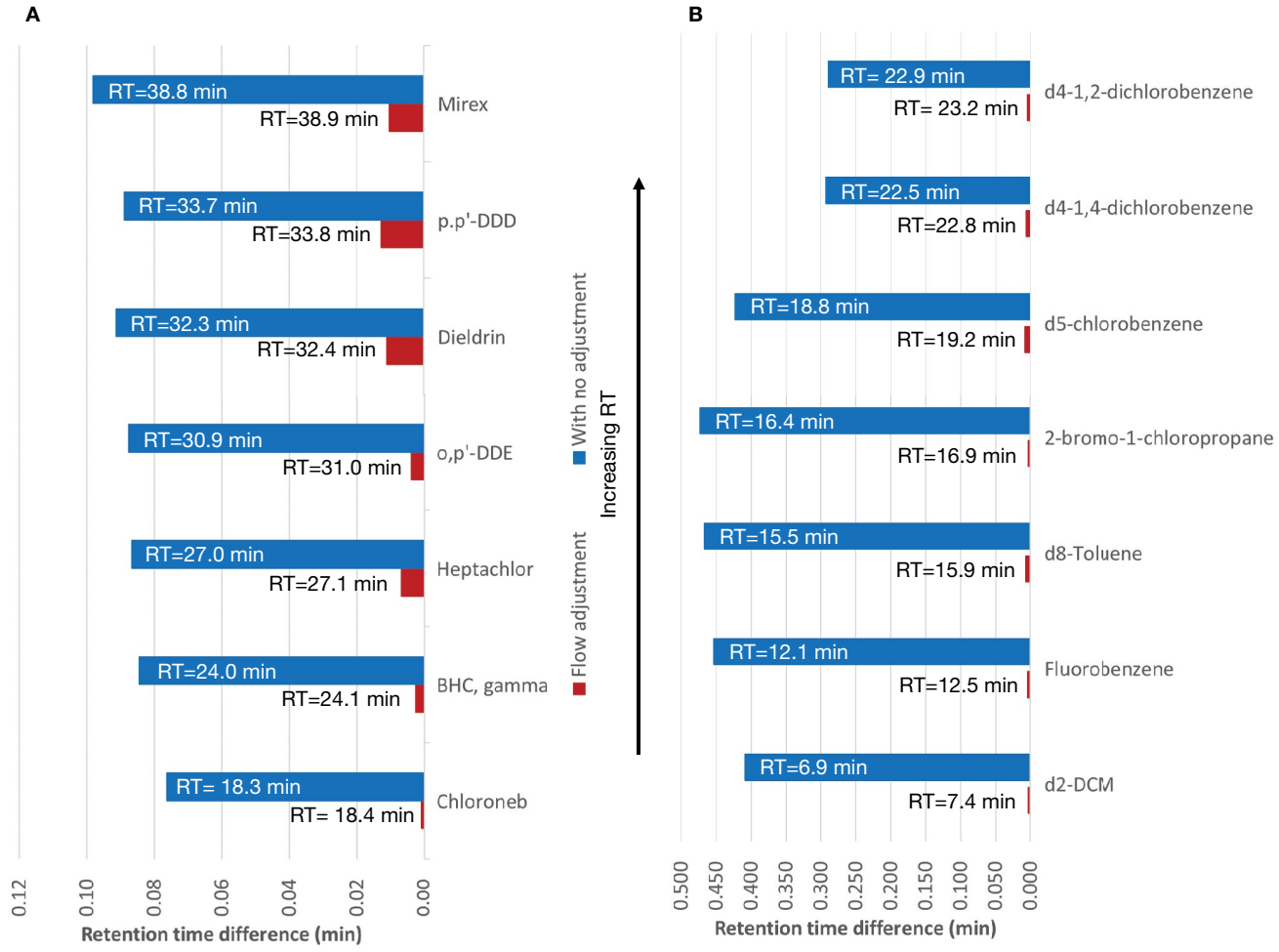

Figure 3. Charts showing the retention time difference with (red) and without (blue) RTA correction based on column flow for selected pesticides (A) and VOCs (B)

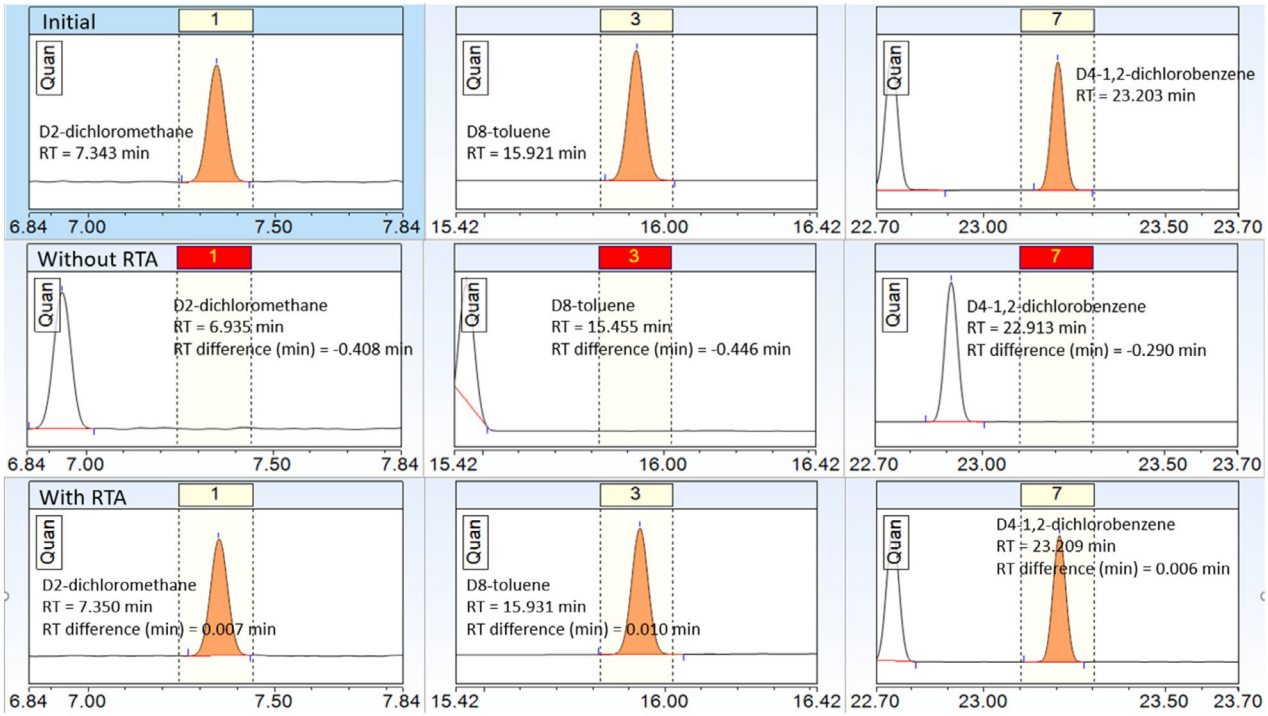

Figure 4. Extracted ion chromatograms (EIC) for D_2-dichloromethane, D_8-toluene and D_4-1,2-dichlorobenzene illustrating the retention time difference before and after trimming the inlet side of the analytical column, with and without flow correction. A ±0.1-minute window labeled with the peak number of the compound is also included.

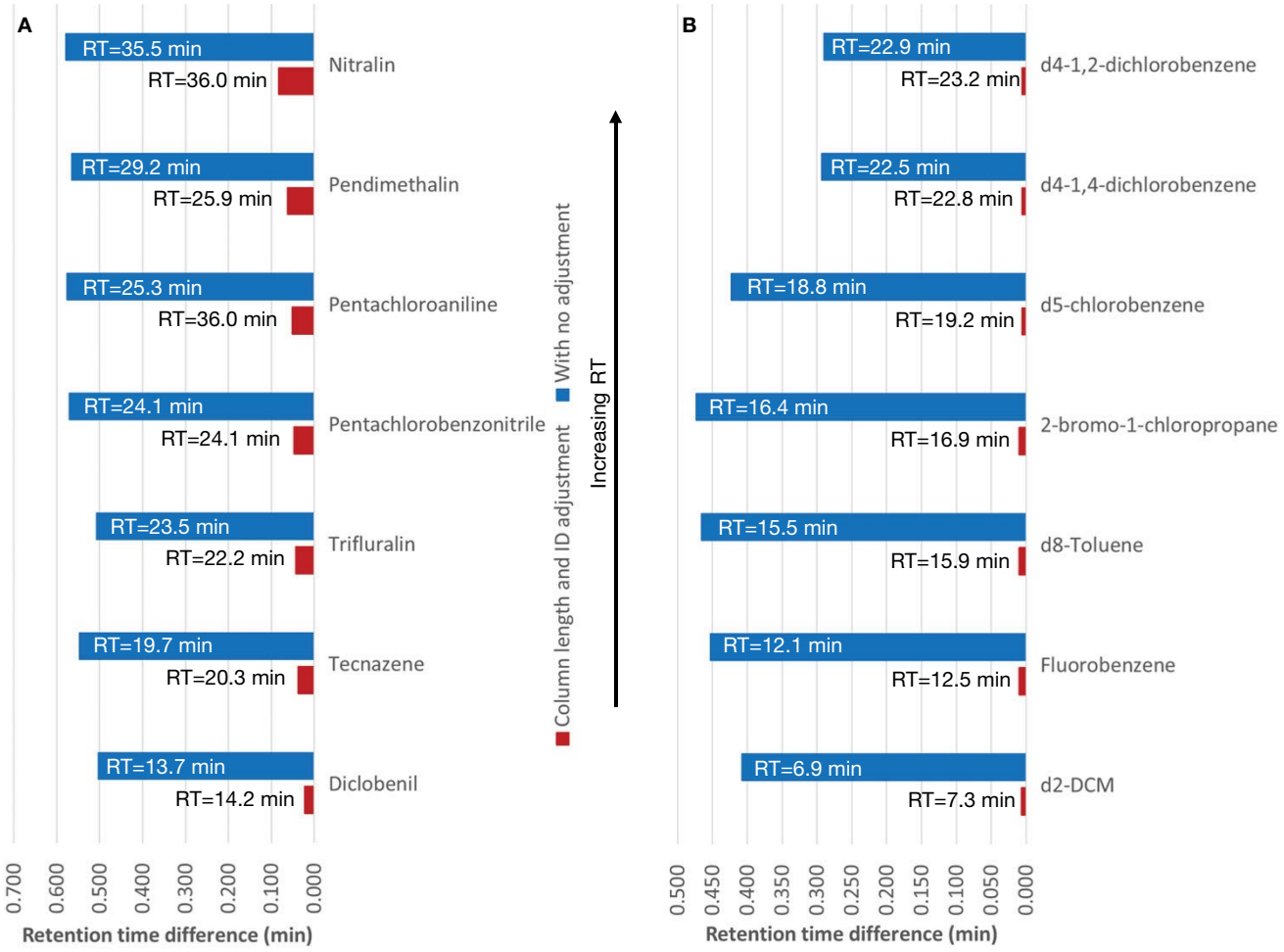

Figure 5. Charts showing the retention time difference with (red) and without (blue) RTA correction based on column dimensions for selected pesticides (A) and VOCs (B)

Conclusions

The data presented in this technical note shows that the Thermo Scientific RTA software corrects for RT drifts easily, and by adjusting the column flow or column dimensions, can ensure that consistent retention times are maintained irrespective of column maintenance, GC/GC-MS system, or sample introduction method. This has been demonstrated for a range of chemicals including VOCs and pesticides, after trimming a significant section from the analytical column, using both headspace sampling and liquid injection. By using the RTA software, users in analytical testing or scientific research laboratories can:

- Quickly and easily ensure RT alignment for either GC or GC-MS analysis in a single isothermal run and based on a single reference compound, regardless of the analytical method or sample composition.
- Analyze pesticides, adjusting retention times to within SANTE guidelines of ±0.1 minute window allowed for retention time difference, after column maintenance.
- Save the time otherwise required to adjust processing methods to ensure that the peak identification is correct after column maintenance or changing columns.
- The Thermo Scientific RTA software provides clear advantages for analytical testing and scientific research laboratories where RT consistency is critical to ensure correct identification of compounds and save time checking and adjusting processing methods.

Reference

1. SANTE/12682/2019. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. Supersedes SANTE/11813/2017. Implemented by 01/01/2020.

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