Journal of Chromatography A, 1373 (2014) 179–189

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

What experimental factors influence the accuracy of retention projections in gas chromatography–mass spectrometry?

Michael B. Wilson[a], Brian B. Barnes[b], Paul G. Boswell[a,*]

[a] Department of Horticultural Science, University of Minnesota, 1970 Folwell Avenue, St. Paul, MN 55108, USA
[b] Department of Chemistry, University of Minnesota, 207 Pleasant Street SE, Minneapolis, MN 55455, USA

ARTICLE INFO

Article history:
Received 6 June 2014
Received in revised form 30 August 2014
Accepted 11 November 2014
Available online 18 November 2014

Keywords:
Gas chromatography–mass spectrometry
Retention prediction
Retention projection
Compound identification
Instrument non-idealities
Programmed-temperature retention from isothermal retention

ABSTRACT

Programmed-temperature gas chromatographic (GC) retention information is difficult to share because it depends on so many experimental factors that vary among laboratories. Though linear retention indexing cannot properly account for experimental differences, retention times can be accurately calculated, or "projected", from shared isothermal retention vs. temperature (*T*) relationships, but only if the temperature program and hold-up time vs. *T* profile produced by a GC is known with great precision. The effort required to measure these profiles were previously impractical, but we recently showed that they can be easily back-calculated from the programmed-temperature retention times of a set of 25 *n*-alkanes using open-source software at www.retentionprediction.org/gc. In a multi-lab study, the approach was shown to account for both intentional and unintentional differences in the temperature programs, flow rates, and inlet pressures produced by the GCs. Here, we tested 16 other experimental factors and found that only 5 could reduce accuracy in retention projections: injection history, exposure to very high levels of oxygen at high temperature, a very low transfer line temperature, an overloaded column, and a very short column ($\leq$15 m). We find that the retention projection methodology acts as a hybrid of conventional retention projection and retention indexing, drawing on the advantages of both; it properly accounts for a wide range of experimental conditions while accommodating the effects of experimental factors not properly taken into account in the calculations. Finally, we developed a four-step protocol to efficiently troubleshoot a GC system after it is found to be yielding inaccurate retention projections.

1. Introduction

Despite continued advances in mass spectrometry, the identification of small molecules from complex mixtures remains a major bottleneck. This is especially true in fields like metabolomics where it is often the goal to identify as many compounds as possible, though samples could easily contain tens of thousands of compounds [1,2]. There, GC–MS is one of the primary analytical tools for identification of volatile and semi-volatile compounds because it offers two independent and complimentary dimensions of information to help identify compounds: mass spectra and GC retention. Though mass spectral information has found wide use for compound identification, GC retention remains underutilized despite its potential utility because it is so difficult to reproduce across laboratories.

GC retention times are essentially irreproducible because they depend on a host of experimental variables that differ among laboratories and instruments. While some of those variables are controllable (temperature program, flow rate, carrier gas, etc.), some are not (temperature calibration errors, inexact column dimensions, flow rate non-idealities, etc.). Therefore, even if standard experimental conditions were universally adopted (which is unlikely), GC retention times would still be irreproducible because of the uncontrollable differences between GC systems.

In order to accommodate some differences in experimental conditions, linear retention indexing was developed [3]. In linear retention indexing, a series of standards are spiked into the sample before it is run in a temperature program. Retention is then reported not as a time, but as an index describing the position a compound elutes between its two bracketing standards. The idea is that, since the standards are subjected to the same experimental conditions as the analytes, linear retention indexing can accommodate some changes in the experimental conditions.

---

* Corresponding author at: 328 Alderman Hall, 1970 Folwell Ave, St. Paul, MN 55108, USA. Tel.: +1 612 250 5188.
E-mail addresses: wils0888@umn.edu (M.B. Wilson), bbbarnes@umn.edu (B.B. Barnes), boswell@umn.edu, bosw0011@umn.edu (P.G. Boswell).

http://dx.doi.org/10.1016/j.chroma.2014.11.030
0021-9673/

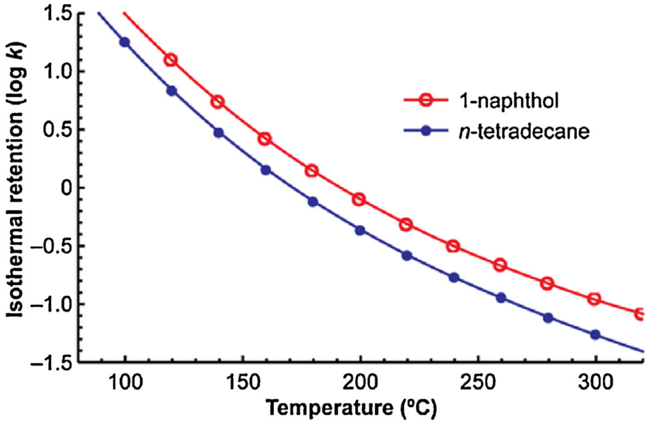

Fig. 1. Isothermal retention vs. *T* relationships of two different compounds, 1-naphthol and *n*-tetradecane.

While linear retention indices are certainly *less* sensitive to changes in experimental conditions than retention times, they are still strongly affected by them [4–6]. Thus, in order for retention indices to be at all reliable, they must be used under precisely the same experimental conditions as they were originally collected (or used under a narrow range of translated methods [7–9]). But even then, small, unintentional differences between GC systems are enough to cause significant differences between the retention indices measured on each system [11]. Some of the unintentional differences between GC instruments can be minimized by retention time locking (specifically, inlet pressure and column length), which requires a user to adjust their inlet pressure until the retention of a standard matches that measured on the original GC [7], but it offers no way to easily calibrate out unintentional errors in the temperature program.

Instead of building a shared database of retention indices, a more general approach is to build a database of isothermal retention factor (k) vs. T relationships (two are shown in Fig. 1). Using these relationships, each compound's programmed-temperature retention time can be calculated using the following equation [12–17] (note that it holds true except in constant flow rate mode under moderate gas decompression [9]):

$$\int_0^{t_R} \frac{\mathrm{d}t}{t_{M,T}(k_T+1)} = 1 \tag{1}$$

where t_R is the retention time of the compound, and $t_{M,T}$ and k_T are the hold-up time and retention factor at temperature T. The equation essentially treats a programmed-temperature run as a series of infinitesimally small isothermal steps that closely approximate the true temperature program. Eq. (1) can be accurately solved for t_R if the following three relationships are precisely known: (1) the isothermal k vs. T relationship for the compound (as in Fig. 1), (2) the T vs. time relationship produced by the GC instrument (the temperature profile), and (3) the t_M vs. T relationship produced by the GC instrument (the hold-up time profile). However, when any of these relationships are more complicated than simple linear functions, Eq. (1) usually has to be solved numerically. To solve it numerically, the temperature program is broken up into a series of very short isothermal steps and in each step, the fraction of the column traveled by the compound is calculated based on its k at the T of that step and the t_M at that T. Using the following summation equation, analogous to Eq. (1), for numerical integration:

$$\sum_{i=1}^{n} \frac{\delta t}{t_{M,T}(k_T+1)} \geq 1 \tag{2}$$

where n is the smallest integer that makes the inequality true, and t_R can be calculated from

$$t_R = \sum_{i=1}^{n} \delta t \tag{3}$$

We call this approach, "retention projection" because temperature-programmed retention times are projected from isothermal retention data. The big advantage of retention projection is that it can theoretically calculate accurate retention times under virtually any temperature program, flow rate, inlet pressure, column length, inner diameter, and film thickness. In fact, there are only two fundamental limitations: the stationary phase and the carrier gas must be the same as those originally used to measure the isothermal k vs. T relationships.

Unfortunately, retention projection is not accurate unless the temperature profile and the hold-up time profile actually produced by the GC are known with great precision. Small imperfections in the temperature calibration, column dimensions, or the flow rate/inlet pressure can cause large errors in projected retention times. Some researchers have successfully taken these imperfections into account by making meticulous measurements of them [13,18], but the amount of effort required is impractical for most users. Moreover, these measurements would have to be re-made every time the experimental conditions are deliberately or inadvertently changed.

Alternatively, a new approach was recently reported to easily measure and account for such imperfections in the temperature and hold-up time profiles [19]. First, one spikes their sample with a set of 25 *n*-alkane samples and subjects it to temperature-programmed elution. Then the retention times of the *n*-alkanes are entered into online software at www.retentionprediction.org/gc. The software uses the retention times of the standards to iteratively back-calculate the effective temperature program and hold-up time profiles that must have been produced in the run to give those retention times. Finally, it uses the back-calculated profiles to project expected retention times for all the other compounds for which isothermal k vs. T relationships have been measured (currently, a database of only 97 compounds is available on the site, but we are in the process of expanding it).

In a multi-lab study [11], the retention projection methodology was shown to account for both intentional and unintentional differences between GC systems in different laboratories, enabling retention times to be calculated with the same level of accuracy regardless of the instrument, temperature program, or inlet pressure/flow rate used. Since the error was laboratory-independent, it was also possible to calculate the appropriate retention time tolerance windows for each projected retention time. This makes it possible to determine the probability of an incorrect peak assignment without needing to have a standard of the compound physically on hand. However, the retention time tolerance windows can only be trusted if one's system is in a "like new" state. Therefore, to ensure the integrity of the calculated tolerance windows, a simple system suitability check was developed [11]: After the software back-calculates the effective temperature and hold-up time profiles, it attempts to project the retention times of 12 chemically diverse test compounds. If error in the projected retention times of these 12 standards is under a certain threshold (which is calculated for a given run based on the temperature program, flow rate/inlet pressure, column dimensions, and film thickness used), the system is deemed suitable and subsequent retention time tolerance windows can be trusted.

The system suitability check has helped us to identify GC systems in an unsuitable state, but once they were identified, it was often unclear what caused them to fail the suitability check. This made us wonder, what factors *could* cause the system suitability