Journal of Chromatography A, 1374 (2014) 207–215

Contents lists available at ScienceDirect

Journal of Chromatography A

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

A practical methodology to measure unbiased gas chromatographic retention factor vs. temperature relationships

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ARTICLE INFO

Article history:
Received 30 August 2014
Received in revised form 7 November 2014
Accepted 10 November 2014
Available online 13 November 2014

Keywords:
Retention projection
Retention prediction
Gas chromatography–mass spectrometry
Retention library
Retention database.

ABSTRACT

Compound identification continues to be a major challenge. Gas chromatography–mass spectrometry (GC–MS) is a primary tool used for this purpose, but the GC retention information it provides is underutilized because existing retention databases are experimentally restrictive and unreliable. A methodology called "retention projection" has the potential to overcome these limitations, but it requires the retention factor (k) vs. T relationship of a compound to calculate its retention time. Direct methods of measuring k vs. T relationships from a series of isothermal runs are tedious and time-consuming. Instead, a series of temperature programs can be used to quickly measure the k vs. T relationships, but they are generally not as accurate when measured this way because they are strongly biased by non-ideal behavior of the GC system in each of the runs. In this work, we overcome that problem by using the retention times of 25 n-alkanes to back-calculate the effective temperature profile and hold-up time vs. T profiles produced in each of the six temperature programs. When the profiles were measured this way and taken into account, the k vs. T relationships measured from each of two different GC–MS instruments were nearly as accurate as the ones measured isothermally, showing less than two-fold more error. Furthermore, temperature-programmed retention times calculated in five other laboratories from the new k vs. T relationships had the same distribution of error as when they were calculated from k vs. T relationships measured isothermally. Free software was developed to make the methodology easy to use. The new methodology potentially provides a relatively fast and easy way to measure unbiased k vs. T relationships.

1. Introduction

The identification of small molecules continues to be a major bottleneck in the analysis of complex mixtures. Typically, only a small fraction of the compounds in a sample can be identified with high confidence, requiring meticulous work by a skilled individual. Of the analytical tools available, gas chromatography–mass spectrometry (GC–MS) is one of the primary tools used for this purpose. It provides two complementary pieces of information that can be used for identification: mass spectra and chromatographic retention information. To identify a compound by GC–MS, one runs samples of potential chemical identities and eliminates ones that have significantly different mass spectra and retention times, ideally leaving only one potential identity remaining.

However, it is often impractical to obtain a sample of every potential chemical identity, so we must rely on shared databases of mass spectral and retention information to make identifications. Though mass spectral databases have found wide use for compound identification, shared GC retention databases have found relatively limited use despite their potential value for compound identification. There are a number of reasons for this. First, in order to reproduce the retention data, one is limited to using precisely the same experimental conditions that were used to build the database [1–3] (or to one of a narrow range of translated methods [4–6]). But even then, it is almost impossible to strictly reproduce the experimental conditions used to develop the database because the retention data are biased by non-ideal behavior of the GC system used to measure them (by "non-ideal" GC system behavior, we mean behavior that deviates from that of an ideal GC system: temperature calibration error, flow rate error, imprecise column dimensions, etc.) [7]. Because of this, it is unclear how much error one should expect if the shared retention data are used across different systems, making it difficult to use shared retention information to reject a potential identity on solid statistical grounds.

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http://dx.doi.org/10.1016/j.chroma.2014.11.018
0021-9673/

Currently, the most common way to share retention data in temperature-programmed GC runs is as linear retention indices (LRIs) [8]. LRIs describe the position a compound elutes between a pair of bracketing standards. Since they are calculated relative to the retention times of two other compounds subjected to the same experimental conditions, the idea is that they should be less sensitive to the small variations in the experimental conditions used to measure them. They are indeed less sensitive to them than absolute retention times, but they are still strongly affected by them. In fact, LRIs are affected by a change in almost any experimental condition: the temperature program, the flow rate/inlet pressure, the outlet pressure, the column length, the inner diameter, and the stationary phase film thickness. Even relatively small non-idealities in those experimental parameters have been found to cause significant shifts [7]. Retention time locking can be used in combination with linear retention indexing to improve its reproducibility across GC systems by calibrating out differences in hold-up time between the two GC systems [4]. However, it provides no way to account for non-idealities in the temperature program and a user is still limited to using precisely the same experimental conditions as were originally used to measure the data (or to one of a narrow range of translated methods).

A far less restrictive way to share GC retention information is to compile a database of isothermal retention factors (k) as a function of temperature. Then, temperature-programmed retention times are calculated by considering the temperature program as a series of very short isothermal steps as in Eq. (1) (which is analogous to the integral, but can be solved with more complicated, nonlinear t_{M} vs. T, T vs. time, and $\ln k$ vs. T relationships) [9–14]:

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

where $t_{\mathrm{M},T}$ is the hold-up time, k_T is the retention factor at the T of the step, and n is the smallest integer that makes the inequality true. 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. Its retention time, t_{R}, is then calculated from the time required for the compound to travel the entire length of the column:

$$t_{\mathrm{R}} = \sum_{i=1}^{n} \delta t \tag{2}$$

We call this approach “retention projection” because temperature-programmed retention times are “projected” from isothermal k vs. T relationships. (Stated another way, the static k vs. T relationships manifest themselves as different retention times when they are “projected” onto different experimental conditions.) The major advantage of this approach is that the k vs. T relationships can be used to calculate a compound’s retention time under a wide range of temperature programs, flow rates/inlet pressures, outlet pressures, and column dimensions. Only the stationary phase and the carrier gas must be fixed. Furthermore, when this approach is combined with a novel back-calculation algorithm to account for GC system non-idealities (see Section 1.1), we have found retention projections to be robust and considerably more accurate than retention indexing when used across laboratories [7]. More importantly, the methodology was found to account for virtually all differences between laboratories and methods, making it possible to calculate the appropriate retention time tolerance window for each projected retention time with a known, absolute level of confidence.

Due to these and other benefits, we considered building a larger database of isothermal k vs. T relationships to make the retention projection methodology more broadly useful for compound identification. The most straightforward way to measure these k vs. T relationships is to directly measure k in a series of isothermal runs over a range of temperatures, however this approach is not practical for large numbers of compounds. First, it takes a long time—if the retention of compounds in a mixture span a wide range, it is necessary to measure retention at 10–15 different temperatures to ensure collection of enough retention factors for both poorly retained and well-retained compounds. Data collection at each temperature takes about 1.5 h to allow the temperature to equilibrate, to make the hold-up time measurements, to run the sample mixture, and to clear out the column at high temperature to prepare it for the next run. Second, a high-accuracy temperature probe and careful annotation of the true temperature for each measurement is required to avoid bias from temperature calibration error, which adds further complication and extra equipment.

Instead of directly measuring k vs. T relationships from a series of isothermal measurements, a faster approach involves running a set of temperature programs and using a compound’s retention time in each run to solve for its k vs. T relationship [15–18]. The solution is found iteratively, by adjusting a k vs. T relationship until the projected retention times in each temperature program are as close as possible to the measured retention times. To constrain the possible solutions, an equation is used to describe the k vs. T relationships. The following thermodynamic relationship has been shown to fit these relationships with good precision [17,19–21]:

$$k = e^{A+B/T+C\ \ln(T)} \tag{3}$$

$$A = \frac{\Delta S(T_0) - \Delta C_p\ \ln(T_0) - \Delta C_p}{R} \tag{4}$$

$$B = -\frac{\Delta H(T_0) - \Delta C_p T_0}{R} \tag{5}$$

$$C = \frac{\Delta C_p}{R} \tag{6}$$

where T is the temperature, T_0 is a reference temperature (here we use 273.15 K), $\Delta H(T_0)$ and $\Delta S(T_0)$ are the changes in molar enthalpy and entropy for transfer of the analyte from the gaseous mobile phase into the stationary phase at the reference temperature, and ΔC_{p} is the change in its isobaric heat capacity for the transfer. Thus, with this equation, three parameters describe a compound’s k vs. T relationship: $\Delta H(T_0)$, $\Delta S(T_0)$, and ΔC_{p}.

While this approach is relatively fast, it can introduce considerable bias into the measurement. In its simplest form, the assumption is usually made that both the temperature profile and the t_{M} vs. T profiles produced by the GC system are ideal, which is rarely the case, thereby introducing bias into the k vs. T relationships. McGinitie et al. [18] recently reported a protocol to measure and account for some system non-idealities. First, the column was rolled out and its precise length was measured. Then the column was rewound, installed, and the t_{M} was measured at three temperatures by injecting methane, which was then used to calculate the column’s effective inner diameter. Then, the Grob test mixture was run under six different temperature programs and a custom MATLAB script was used to iteratively solve for the effective film thickness. Finally, sets of six temperature programs were run to iteratively solve for values of $\Delta H(T_0)$, $\Delta S(T_0)$, and ΔC_{p} of individual compounds using the above measurements of column length, effective inner diameter, and effective film thickness using another custom MATLAB script.

While the protocol described by McGinitie et al. made a strong attempt to account for bias resulting from the column, in our view, it is not a viable solution. First, the amount of effort and expertise required is substantial. A typical GC user is unlikely to use such a methodology to calibrate their system. The solution is also incomplete; it does not account for temperature inaccuracy and it assumes that the column inlet and outlet pressures are ideal. Of course, they could be taken into account by careful measurement,