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Easy and accurate calculation of programmed temperature gas chromatographic retention times by back-calculation of temperature and hold-up time profiles

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ABSTRACT

Linear retention indices are commonly used to identify compounds in programmed-temperature gas chromatography (GC), but they are unreliable unless the original experimental conditions used to measure them are stringently reproduced. However, differences in many experimental conditions may be properly taken into account by calculating programmed-temperature retention times of compounds from their measured isothermal retention vs. temperature relationships. We call this approach "retention projection". Until now, retention projection has been impractical because it required very precise, meticulous measurement of the temperature vs. time and hold-up time vs. temperature profiles actually produced by a specific GC instrument to be accurate. Here we present a new, easy-to-use methodology to precisely measure those profiles: we spike a sample with 25 *n*-alkanes and use their measured, programmed-temperature retention times to precisely back-calculate what the instrument profiles must have been. Then, when we use those back-calculated profiles to project retention times of 63 chemically diverse compounds, we found that the projections are extremely accurate (e.g. to ±0.9 s in a 40 min ramp). They remained accurate with different temperature programs, GC instruments, inlet pressures, flow rates, and with columns taken from different batches of stationary phase while the accuracy of retention indices became worse the more the experimental conditions were changed from the original ones used to measure them. We also developed new, open-source software (http://www.retentionprediction.org/gc) to demonstrate the system.

1. Introduction

Gas chromatography–mass spectrometry (GC–MS) is an essential tool in fields such as metabolomics that seek to identify as many compounds as possible in complex mixtures. While the MS information acquired from GC–MS has found especially wide use in compound identification, the GC retention information, despite its high information content, is severely underutilized because it is so difficult to reproduce or transfer between instruments. This is particularly true for programmed-temperature runs, which are by far the most common form of GC, especially in metabolomics.

The reproducibility of GC retention times is poor because they depend on so many experimental factors. Some factors are controllable (e.g. temperature program, flow rate, column make/model, etc.) and some are not easily controlled (e.g. oven calibration errors, flow rate errors, column dimensions, etc.) and may even vary from run to run. One can imagine that controllable factors might be eliminated by defining a set of rigidly adhered to standard experimental conditions for running programmed-temperature GC experiments, but no such standards have been widely adopted and are unlikely to be. Yet even if all controllable experimental conditions could be held constant, retention times would still vary due to the uncontrollable factors—just changing the make/model of GC instrument can cause major shifts in retention times.

Retention indexing was first introduced by Kováts [1] as a metric to describe isothermal retention that is far less sensitive to small perturbations in experimental conditions. In retention indexing, samples are spiked with a set of *n*-alkane standards before they are run. Then, retention is reported as a retention index, I_x, which describes the time a compound *x* elutes relative to the nearest two bracketing *n*-alkane standards:

$$I_x = 100C_n + 100\frac{\log\ t'_{R(x)} - \log\ t'_{R(n)}}{\log\ t'_{R(n+i)} - \log\ t'_{R(n)}} \quad (1)$$

where $t'_{R(x)}$ is the adjusted retention time of compound *x*, $t'_{R(n)}$ and $t'_{R(n+i)}$ are the adjusted retention times of *n*-alkane standards

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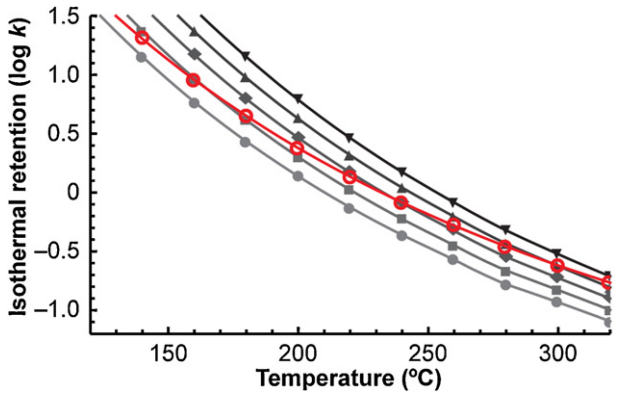

Fig. 1. Isothermal retention ($\log k$) vs. T relationships for n-heptadecane (●), n-octadecane (■), n-nonadecane (◆), n-eicosane (▲), n-heneicosane (▼), and anthracene (○). The retention index of anthracene is highly dependent on the temperature at which it is measured.

eluting before and after compound x, and C_n and C_{n+i} are the number of carbon atoms in those standards. Because the bracketing standards are in the same run and therefore experience the same experimental conditions as the sample compounds, they can accommodate differences in the flow rate and column dimensions, along with small temperature fluctuations and some differences in the amount of stationary phase, as long as the same type of stationary phase material is used.

Later, van Den Dool and Dec. Kratz [2] extended the concept of retention indexing to programmed-temperature runs. In analogy to Eq. (1), they defined the linear retention index (LRI) as

$$\mathrm{LRI}_x = 100C_n + 100\frac{t_{\mathrm{R}(x)} - t_{\mathrm{R}(n)}}{t_{\mathrm{R}(n+1)} - t_{\mathrm{R}(n)}} \quad (2)$$

where $t_{\mathrm{R}(x)}$ is the retention time of compound x, and $t_{\mathrm{R}(n)}$ and $t_{\mathrm{R}(n+1)}$ are the retention times of n-alkane standards eluting before and after compound x. Today, the LRI is the most widely used metric to catalogue retention data; the NIST 08 database contains 240,695 retention indices for 42,888 compounds of which 70% are LRIs [3].

Unfortunately, LRIs measured under different experimental conditions are *not the same*. Fig. 1 shows why. The isothermal retention (in terms of $\log k$) vs. T relationships for 5 different n-alkane standards are shown along with that of anthracene. Linear retention indexing assumes that anthracene should always elute at the same position between the two bracketing n-alkanes, but that is clearly not the case. For example, at 320 °C, anthracene does not even elute between the same two n-alkanes as it does at 140 °C. In fact, it does not elute between the next pair of standards or even the next pair after that—*it elutes between the pair of n-alkanes three CH_2 units longer than expected*. Clearly this is due to a large difference in the enthalpy of transfer between anthracene and the bracketing n-alkanes.

LRIs are plagued by the same fundamental problem in programmed-temperature runs, where they are not only dependent on the specific temperature program used to measure them, but since a change in the flow rate affects the "program steepness" of a temperature ramp [4], LRIs also depend on the particular flow rate/inlet pressure, the mode of gas flow regulation (constant inlet pressure vs. constant flow rate), and the column dimensions used. Moreover, if imperfections in the GC instruments cause large enough differences in these factors between various makes/models of GC instruments, LRIs will also be dependent on the specific GC instrument used to measure them. Several methods have been reported that enable the transfer of LRIs measured under one set of experimental conditions to a different set with better agreement [5–7], but even then, their accuracy remains questionable unless the experimental conditions originally used to measure them are *rigorously* reproduced [8,9].

A different approach, called "method translation" [10–12] (of which Retention Time Locking, or RTL, is a less general implementation [11]), enables one to predict retention even after changing the temperature program, the flow rate/inlet pressure, and the column dimensions, *so long as they are changed together in such a way that*

(a) for each heating ramp in the temperature program, the product of the heating rate (r) and the hold-up time (t_{M}), also called the normalized heating rate, remains unchanged, and
(b) for each temperature plateau (of length t_{p}) in the temperature program, the ratio $t_{\mathrm{p}}/t_{\mathrm{M}}$ remains unchanged.

When these two conditions are met, the retention pattern remains the same, allowing retention to be translated to different, but related sets of experimental conditions.

While method translation represents a theoretically sound and therefore more accurate approach than retention indexing, it too has a number of limitations: First, it requires that program steepness be held constant, which *greatly* restricts the range of allowable experimental conditions. It also makes it impossible to use program steepness as a way to alter the selectivity of a separation. Perhaps even more importantly, method translation assumes that the temperature program produced by the GC oven is ideal—it cannot correctly account for any non-idealities in the actual T vs. time profiles produced by a given GC oven. Obviously it cannot account for differences between different makes/models, though it could be combined with retention indexing to accommodate them to some extent.

Another more general approach involves *calculating* retention times in a programmed-temperature experiment from the measured isothermal retention vs. T relationships of each compound (as shown in Fig. 1). We call this "retention projection" because the retention times in a temperature program are *projected* from the isothermal retention measurements. The general equation used to project retention times in a programmed temperature run has been described previously [13,14]:

$$\int_0^{t_{\mathrm{R},x}} \frac{dt}{t_{\mathrm{M},T}(k_{x,T}+1)} = 1 \quad (3)$$

where $t_{\mathrm{R},x}$ is the retention time of the compound x, $t_{\mathrm{M},T}$ is the hold-up time at temperature T, and $k_{x,T}$ is the retention factor of compound x at temperature T. The equation treats a programmed-temperature run as the sum of a series of infinitesimally small isothermal temperature steps that, taken together, closely approximate the true temperature program. It can be accurately solved for t_{R} [15] if the following three relationships are precisely known: (1) the isothermal k vs. T relationship for compound x, (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).

Unlike retention indexing and method translation, retention projection has the potential to accurately predict retention times with virtually any temperature program, flow rate/inlet pressure, film thickness, carrier gas type, and column dimensions, so long as the stationary phase material is chemically identical to that used to establish the database, because it accounts for the differences in a theoretically sound way. In a retention projection system, users can calculate retention times of compounds from their measured isothermal k vs. T relationships (which would be stored in a central database) along with the temperature profile and hold-up time profile produced by their instrument. In fact, several researchers [13,15–20] have attempted to use retention projection to predict

retention times in programmed-temperature runs, but *unless the temperature and hold-up time profiles produced by the GC instrument are known with great precision, the accuracy of retention projections suffer*. In one report, Vezzani et al. [15] found that even when the hold-up time profile was precisely measured, errors in the calibration of their GC oven of less than 1 °C (affecting the accuracy of the temperature profile they used in their calculations) were enough to cause considerable error in retention projections. This remains an important problem as modern GC ovens are specified with oven temperature accuracies of ±3–5 °C (not to be confused with temperature "precision" or "resolution", which are often specified to 0.01 °C). Even if a GC oven could produce a perfect temperature profile, the hold-up time vs. T profile would still need to be measured with high precision as it is strongly affected by differences in column length, inner diameter, and different temperature zones along the length of the column (e.g. the transfer line between the oven and a mass spectrometer source). This makes retention projection highly impractical because the average GC user finds it impractical to make such meticulous, time-consuming measurements every time they want to predict retention times.

We present a new methodology that solves this problem: We simply spike our sample with a set of *n*-alkane standards and run the sample under programmed-temperature elution. Then we use the retention times of the *n*-alkane standards to *back-calculate what the effective temperature and hold-up time profiles were to produce those retention times*. As will be shown herein, the back-calculated profiles can then be used to project the retention times of *other* compounds with extremely high accuracy. This approach is very simple for the user as it requires no more experimental effort than conventional retention indexing.

Based on the success of past work [21,22] in which we applied a similar methodology to project retention in high-performance liquid chromatography, we sought to determine its value for gas chromatography. In this work, we briefly discuss the back-calculation methodology and then compare the accuracy of retention projection with retention indexing under a variety of experimental conditions using a set of 63 chemically diverse compounds. We compare its accuracy with different temperature programs, different flow rates/inlet pressures, different modes of gas flow regulation, different makes/models of GC instruments, and with different batches of DB-5MS UI stationary phase. We also present a new public website (http://www.retentionprediction.org/gc) and open-source software that anyone can use to try out the new GC retention projection system.

2. Experimental

2.1. Compound selection

63 chemically diverse compounds and 25 *n*-alkanes (C_7–C_{26}, C_{28}, C_{30}, C_{32}, C_{34}, and C_{36}) were used in this work. The compounds are shown in Table 1. The 63 compounds were selected to elute over a wide range of retention times and to represent the 5 types of interactions most common in GC as represented by the Abraham descriptors [23–27]. There are compounds that are strong hydrogen bond donors (e.g. 37, 38, 42, 49, 54, and 55) and hydrogen bond acceptors (e.g. 39, 40, 48, 50, 51, 52, 56, 57, 60, 62, and 63), compounds with large excess molar refraction, that is, they interact by pi and/or lone pair interactions (e.g. 41, 42, 43, 45, 46, and 47), compounds that interact by dipole-dipole and dipole-induced dipole interactions (e.g. 33, 44, 48, and 58), and compounds that vary widely in their gas-liquid partition coefficients (e.g. homologous series 1–6, 7–15, 16–22, and 27–32) [28,29]. All chemicals and solvents were purchased from Sigma–Aldrich (St. Louis, MO), Alfa Aesar (Ward Hill, MA), or TCI America (Portland, OR).

2.2. Instrumentation/columns

Three GC–MS instruments were used in this work: (1) a Hewlett Packard (HP, Palo Alto, CA) Model 5890 Series II GC equipped with an HP 5970 single quadrupole mass spectrometer, (2) a Thermo Scientific (Waltham, MA) Trace GC Ultra equipped with a Thermo TSQ Triple Quadrupole mass spectrometer, and (3) an Agilent Technologies (Santa Clara, CA) 7890A GC equipped with a Waters Corporation GCT Premier time-of-flight mass spectrometer. We used He carrier gas, deactivated, straight quartz liners (2 mm inner diameter) containing deactivated quartz wool, an inlet temperature of 290 °C, and a transfer line temperature of 320 °C.

Five DB-5MS UI columns (30 m long, 0.25 mm inner diameter, 0.25 μm film thickness) were used. Three of the columns contained the same batch of stationary phase (batch A) and two of the columns contained stationary phase from different batches (batches B and C).

2.3. Isothermal retention measurements

Compounds 1–63 and the 25 *n*-alkane standards were split into 8 ethyl acetate, benzene, or hexanes solutions (each compound at 500 μM). The isothermal retention factor of each compound was measured at 20 °C intervals from 60 °C to 320 °C. Before and after each run, t_M was measured (at the same T as the run) by injecting air and monitoring the N_2 peak. For both air and sample injections, we injected 1 μL with a split flow of 30 mL/min and a septum purge flow of 10 mL/min. The isothermal retention factors for each compound (measured from the apex of each peak; the software readout gave three decimal places) were then calculated from:

$$k_{x,T} = \frac{t_{R,x}(T) - t_{M,x}(T)}{t_{M,x}(T)} \quad (4)$$

where $t_{M,x}(T)$ is the hold-up time and $t_{R,x}(T)$ is the retention time of compound x at T. The measured values are shown in Table S-1. Retention factors were repeatable to ±0.042%.

2.4. Programmed temperature runs

Compounds 1–63 and the 25 *n*-alkane standards were mixed into one 60% hexanes, 20% ethyl acetate, and 20% benzene solution (each compound at 100 μM). The programmed temperature runs used 1 μL injections, a split flow of 10 mL/min, and a septum purge flow of 10 mL/min. The MS scan rate was 1.87 Hz. The programmed-temperature retention times (measured from the apex of each peak) in each run are shown in Table S-2.

2.5. Software

The new GC retention projection software was compiled for compliance with the Java 1.6 (Oracle, Redwood Shores, CA) runtime environment. It includes the Java OpenGL (JOGL) binding library version 1.1.1 (JogAmp, http://jogamp.org), JavaHelp version 2.0.05 (Oracle, Redwood Shores, CA), and SwingX library version 1.6.2 (Oracle, Redwood Shores, CA). The source code may be downloaded from www.retentionprediction.org/gc/development.

3. Results and discussion

We begin with a brief explanation of how we back-calculate effective temperature and hold-up time profiles and then we compare the accuracy of retention projection (calculated using

Table 1
Measured and predicted retention times in a 26 °C/min ramp.[a]

Compound #	Compound name	Measured t_R (min)	Error in projected t_R w/back-calculated profiles (min)	Error in projected t_R w/assumed profiles (min)	Calculated t_R from NIST RIs (min)
1	Propyl acetate	2.634	−0.003	−0.281	−0.237
2	Butyl acetate	3.980	0.003	−0.413	0.193
3	Pentyl acetate	6.289	−0.001	−0.351	0.052
4	Hexyl acetate	7.860	−0.002	−0.286	−0.015
5	Octyl acetate	9.877	−0.002	−0.297	−0.029
6	Decyl acetate	11.237	0.000	−0.296	−0.007
7	2-Hexanone	3.553	0.000	−0.373	−0.067
8	2-Heptanone	5.818	−0.003	−0.417	−0.218
9	2-Octanone	7.565	0.001	−0.291	0.125
10	2-Nonanone	8.810	0.000	−0.289	0.017
11	2-Decanone	9.751	0.002	−0.295	0.024
12	2-Undecanone	10.515	0.003	−0.297	0.036
13	2-Dodecanone	11.176	−0.001	−0.300	0.056
14	2-Tridecanone	11.771	−0.001	−0.294	0.056
15	2-Pentadecanone	12.828	0.001	−0.281	−0.005
16	Acetophenone	8.593	0.000	−0.297	0.134
17	Propiophenone	9.589	0.000	−0.302	
18	Butyrophenone	10.301	−0.001	−0.306	0.098
19	Valerophenone	11.022	0.000	−0.305	0.057
20	Hexanophenone	11.660	−0.002	−0.302	
21	Heptanophenone	12.240	−0.008	−0.302	
22	Octanophenone	12.783	−0.005	−0.293	
23	Tri-*n*-hexylamine	12.970	−0.004	−0.283	−0.043
24	Tri-*n*-octylamine	15.299	−0.004	−0.247	−0.019
25	Tri-*n*-decylamine	17.634	−0.009	−0.225	−0.046
26	Aniline	7.394	−0.003	−0.308	0.171
27	4-Ethylaniline	9.604	0.004	−0.299	
28	4-Butylaniline	11.093	−0.002	−0.307	
29	4-Hexylaniline	12.300	−0.010	−0.305	
30	4-Octylaniline	13.347	−0.002	−0.286	
31	4-Decylaniline	14.279	−0.001	−0.271	
32	4-Dodecylaniline	15.120	−0.005	−0.254	
33	4-Nitroaniline	12.497	−0.012	−0.310	
34	Toluene	3.303	0.003	−0.344	0.021
35	Ethylbenzene	5.214	−0.002	−0.523	0.252
36	*n*-Butylbenzene	8.467	0.001	−0.294	0.162
37	Phenol	7.351	−0.003	−0.297	
38	Linalool	8.912	0.002	−0.289	0.012
39	*N*,*N*-dimethylisobutyramide	7.285	0.005	−0.295	
40	*N*,*N*-diethylacetamide	7.653	−0.002	−0.296	
41	Naphthalene	9.837	0.001	−0.310	0.237
42	1-Naphthol	11.992	−0.016	−0.319	0.127
43	Anthracene	13.593	−0.005	−0.300	0.458
44	Coumarin	11.621	−0.005	−0.313	0.284
45	Indole	10.619	−0.001	−0.310	−0.063
46	Indole-3-acetic acid ethyl ester	13.803	−0.008	−0.289	
47	Triphenylmethane	14.380	−0.008	−0.282	
48	Benzamide	10.837	−0.009	−0.317	0.001
49	Resorcinol	10.394	−0.012	−0.312	
50	Pyridine	3.009	−0.014	−0.329	0.046
51	4-Methylpyridine	5.213	−0.011	−0.532	0.347
52	*Tert*-butylpyridine	8.882	0.004	−0.295	
53	1-Octanol	8.581	0.003	−0.284	−0.009
54	Octanoic acid	9.503	−0.016	−0.305	−0.024
55	Decanoic acid	10.936	−0.018	−0.315	−0.177
56	Dextromethorphan	15.096	−0.015	−0.271	0.430
57	Lidocaine	13.831	−0.006	−0.287	0.177
58	Caffeine	13.676	−0.007	−0.293	0.206
59	(1*S*,2*R*)-1-phenyl-2-(1-pyrrolidinyl)-1-propanol	12.935	−0.013	−0.305	
60	Diphenylamine	12.601	−0.013	−0.308	0.077
61	Benzocaine	12.313	−0.015	−0.310	
62	Nikethamide	12.115	−0.016	−0.315	
63	Nicotine	11.003	−0.007	−0.315	0.096
Standard deviation of prediction error:[b]			±0.44 s	±19 s	±9.9 s
Average absolute error:			0.32 s	19 s	4.7 s
Median absolute error:			0.18 s	18 s	1.4 s

[a] 5 min at 60 °C, 26 °C/min ramp to 320 °C, hold 15 min.
[b] Standard deviation in predicted retention times from experimental retention times for all solutes.

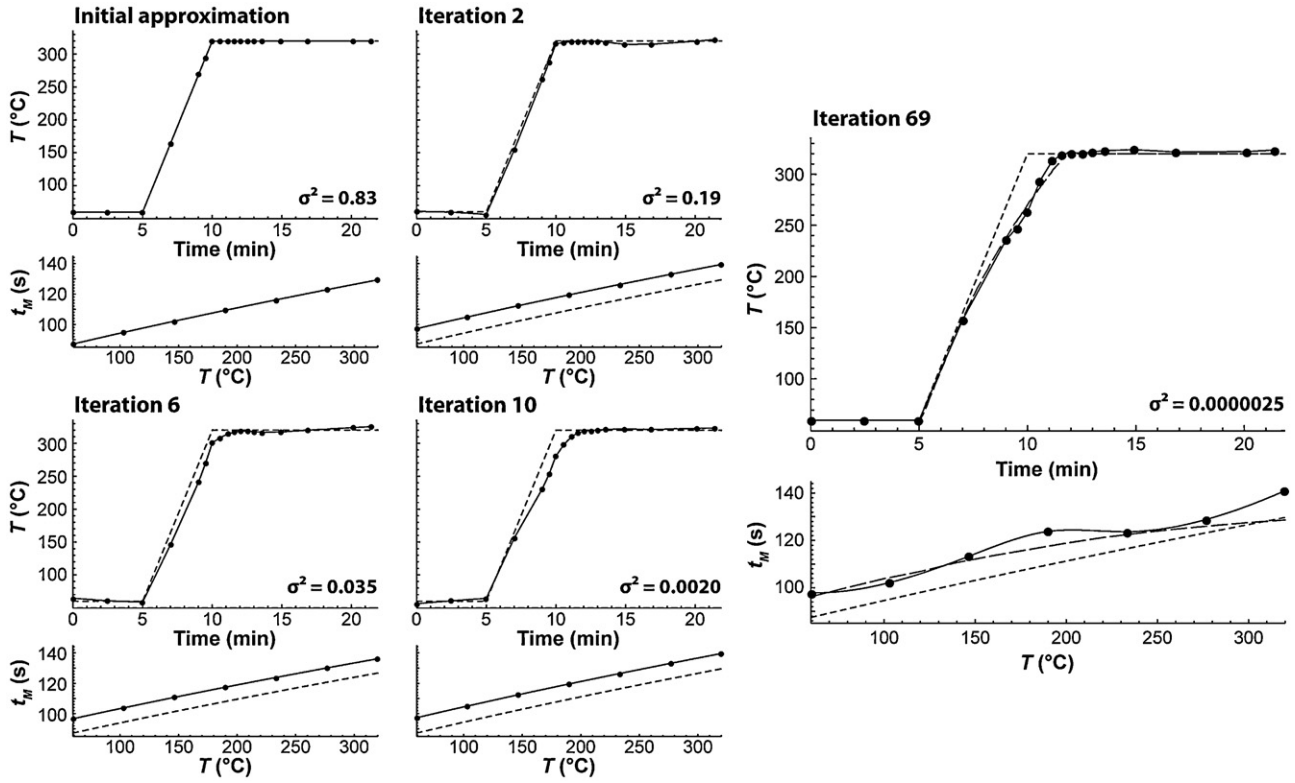

Fig. 2. Selected iterations that show the progression of the back-calculation of a 52 °C/min ramp going from 60 °C to 320 °C, initially held at 60 °C for 5 min. The dotted lines show the ideal temperature and hold-up time profiles, the solid lines show the working profiles under optimization, and the dashed lines in the final iteration show the actual profiles that we measured. The back-calculated profiles of the final iteration more closely match the measured profiles than the ideal ones.

back-calculated profiles and ideal profiles) with retention indexing under a range of experimental conditions.

3.1. Back-calculation of temperature and hold-up time profiles

In the new algorithm, effective temperature and hold-up time profiles are back-calculated using an iterative optimization process similar to one we developed previously [22] for high-performance liquid chromatography. Fig. 2 shows several iterations from the back-calculation of a 52 °C/min ramp going from 60 °C to 320 °C. The algorithm begins with an initial approximation of the two profiles; the working temperature profile is set equal to the programmed one and the working hold-up time profile is calculated [10] from user-specified experimental parameters (see Supporting information). Using those initial profiles, the retention times of the *n*-alkane standards are projected and compared to the actual, measured retention times of the *n*-alkane standards in the run. Then a small change is made to the temperature or hold-up time profile and the retention times of the *n*-alkane standards are re-projected with the new profiles. If the change decreases the error in their projected retention times, the change is kept, otherwise it is rejected. In successive iterations, the algorithm continues making changes to the profiles and re-projecting the retention times of the *n*-alkane standards until further improvements in the accuracy of the projected retention times become insignificant. At that point, the working profiles are considered to be fully optimized. See Supporting information for a more detailed description of the back-calculation algorithm.

We find that back-calculated profiles generally reflect the true profiles produced by the instrument more closely than the ideal (expected) ones. For example, the GC instrument used for Fig. 2 could not heat the oven fast enough to keep up with the 52 °C/min ramp rate, so the temperature profile produced by the instrument lagged the ideal one it was supposed to produce. This lag is reflected in the back-calculated temperature profile (solid line), which more closely resembles the measured profile (dashed line) than the actual one (dotted line). Similarly, the back-calculated hold-up time profile more closely matches the measured hold-up profile than the ideal one. Of course, this is an extreme example—most people will not use their GC oven in a ramp that is too steep for it to produce—but it shows how the algorithm is capable of determining the profile actually produced by a user's GC instrument even under unusual circumstances.

The new GC back-calculation algorithm is part of a web-based, open-source application available at http://www.retentionprediction.org/gc (see Fig. 3). It takes the user through 5 steps:

Step #1: The user enters the make/model of column they used and its length (DB-5MS UI, 0.25 mm inner diameter, 0.25 μm film thickness is currently the only option).
Step #2: The user enters the inlet pressure/flow rate, whether the column outlet is under vacuum or at ambient pressure, and the temperature program used in the run.
Step #3: The user enters the retention times of all the *n*-alkane standards measured in their run.
Step #4: The software back-calculates the effective temperature and hold-up time profiles produced by their instrument in the run.
Step #5: The back-calculated profiles are used to project the retention times of the 63 other compounds for which we have measured isothermal *k* vs. *T* relationships on the DB-5MS UI column.

3.2. Retention projection using back-calculated profiles

First, we discuss retention projection and compare its accuracy with that of retention indexing in a representative 26 °C/min

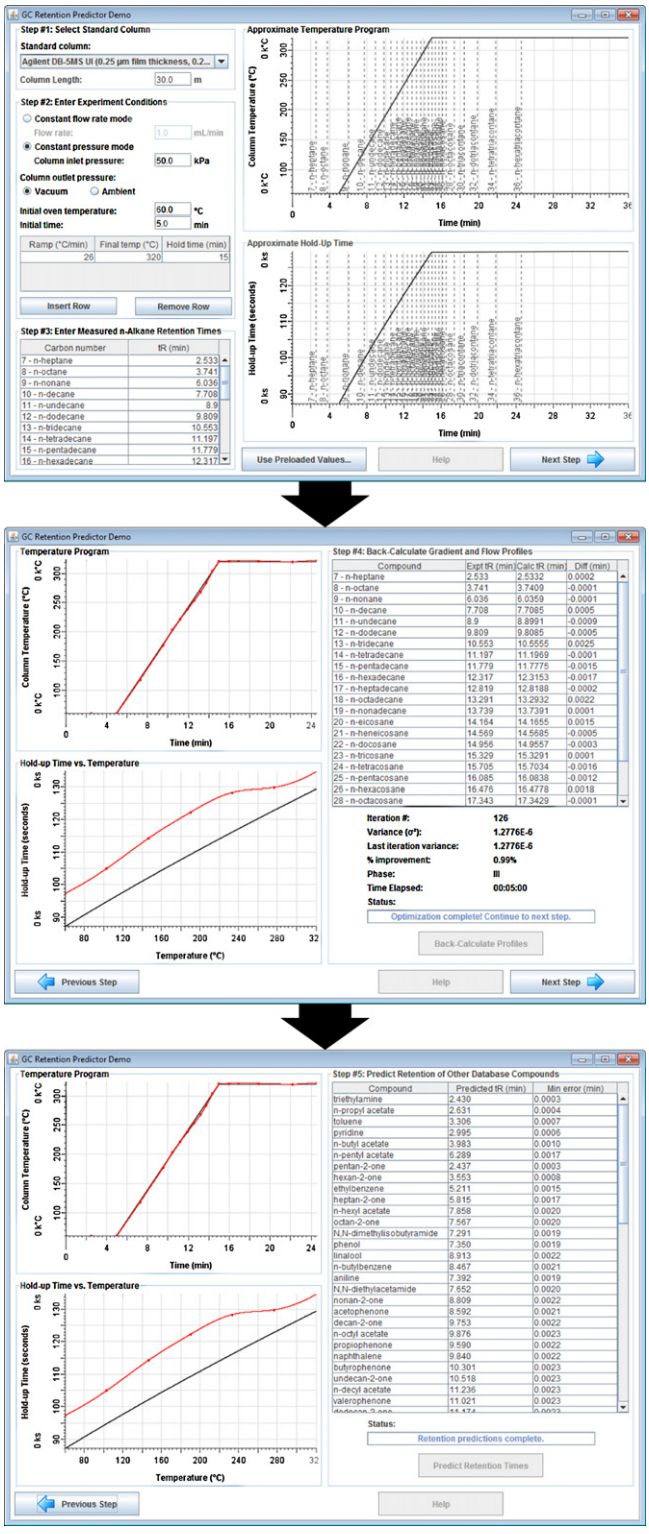

Fig. 3. The GC retention projection application takes the user through 5 steps. The first window shows steps 1–3, the second window shows step 4, and the third window shows step 5 of the retention projection process.

temperature ramp, then with different temperature programs, different makes/models of GC instruments, different inlet pressures, different flow rates, different modes of flow regulation, and with different batches of the same stationary phase.

3.2.1. *Retention projections in a representative 26 °C/min temperature ramp*

A mixture containing compounds 1–63 (spiked with the 25 *n*-alkane standards) was run in a 26 °C/min ramp from 60 °C to 320 °C. The total ion chromatogram of that run is shown in Fig. 4 along with dashed lines showing the retention times that were projected for each compound using the back-calculated profiles. The errors in the projected retention times, along with the measured retention times, are shown in Table 1 in the fourth and third columns, respectively. Overall, the error in those retention projections was only ±0.44 s (±0.007 min), which is near the minimum level of error that could possibly be achieved (±0.2 s or ±0.003 min) given the isothermal retention repeatability and the detector sampling period (see Supporting information). Evidently the back-calculated profiles enable retention times to be projected with an accuracy that is only twofold poorer than the best that can be expected.

Of the 63 compounds, those that exhibit especially strong hydrogen bond, pi/lone pair, or dipole interactions tended to have the largest error in their retention projections. Retention projections for octanoic acid (54) and decanoic acid (55) had the largest errors relative to the other compounds, both compounds being very strong hydrogen bond donors, but *even their retention times were projected with ≤1.1 s error*.

On the other hand, if retention times are projected from the "ideal" (assumed) profiles instead of the back-calculated ones, they are far less accurate. These are shown in the fifth column of Table 1. With a perfectly ideal GC, the temperature profile would precisely equal the programmed one, and the ideal hold-up time profile for an open, round column could be closely approximated with known equations (see Supporting information) [10]. When those assumed profiles were used to project retention in the same 26 °C/min ramp, the overall error was ±19 s, 43-fold worse than when the back-calculated profiles were used.

The last column of Table 1 shows retention times calculated using LRIs taken from the NIST 08 database [3]. We found LRIs that were determined with (5%-phenyl)-methylpolysiloxane columns for 45 of the 63 compounds. Most were measured on a DB-5 or DB-5MS column with the same dimensions and film thickness we used, but other experimental conditions varied because the database generally did not contain LRIs measured under the same conditions we used. Of those 45 LRIs, we removed three outliers that were clearly wrong. The error in retention times determined from the remaining 42 LRIs was ±9.9 s, while the error in retention projections among the same 42 compounds was only ±0.42 s (24-fold more accurate).

One might argue that this is not a fair test of the accuracy of retention indexing as we are only using one retention index in our calculations. Indeed, if enough LRIs were measured for each compound under a wide range of normalized heating rates, there are a number of approaches that could be taken to more closely approximate retention with any given normalized heating rate [30–33] and even under more complicated, multi-step temperature programs [34,35]. This would certainly improve the accuracy of retention indexing, but it nullifies the one advantage retention indexing has over the retention projection system described in this manuscript, namely that it requires less experimental effort to build a library of retention information (it only requires *one* measurement of retention as opposed to several). Moreover, the multiple LRI approach has a major disadvantage compared to retention projection: it would have to assume that a user's GC oven always produces the ideal temperature program, that the flow rate/inlet pressure is ideal, and that the column dimensions are precisely known. To some extent, it could accommodate small non-idealities in each of those factors, *but since it cannot account for them in a theoretically sound way, its accuracy is fundamentally limited*. One could make manual adjustments to their instrumentation or method to offset

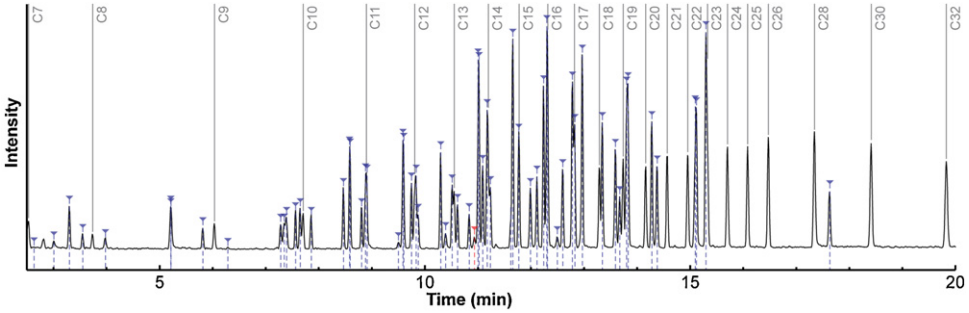

Fig. 4. Total ion chromatogram of an injection containing compounds 1–63 and the 25 *n*-alkanes in a 26 °C/min ramp going from 60 °C to 320 °C. Projected retention times are indicated with dashed, blue lines and the *n*-alkane standards are indicated with solid gray lines. The dashed, red line shows the retention projection for decanoic acid, which had the most error (1.1 s). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

these non-idealities, but it would require considerable experimental effort and may need to be repeated frequently.

3.2.2. Retention projection with different temperature programs

To compare the accuracy of retention projection and retention indexing with different temperature programs, we ran the spiked mixture of 63 compounds in four different temperature ramps: 52 °C/min, 26 °C/min, 13 °C/min, and 6.5 °C/min, all from 60 °C to 320 °C, and in three more complicated, non-linear ramps. We then back-calculated the effective temperature and hold-up profiles in each run (Fig. 5 and the final iteration in Fig. 2). The back-calculated temperature profiles closely resemble the shapes of the ideal temperature profiles, even in the case of the three more complicated temperature programs, and the back-calculated hold-up time profiles resemble the measured hold-up time profiles more closely than the ideal ones.

Using those back-calculated profiles, we projected retention times for the 63 compounds in each run. The overall accuracies of those projections are shown in Table 2, Part A. In each case, the error is extremely low, ranging from ±0.36 s to ±1.0 s. This low error represents only a very small fraction of the ramp time. In the 6.5 °C/min ramp (run **4**), the retention projection error (±1.0 s) represents the lowest percentage of the ramp time—*only 0.042% of 40 min*. With error that represents such a small fraction of the run time, GC retention information alone could become a powerful tool for compound identification. If it were combined with mass spectral information, the identification power of GC–MS could be very improved greatly [21].

Table 2 also shows the accuracy of projected retention times calculated with the ideal temperature and hold-up profiles rather than the back-calculated ones. The accuracy of those retention projections are all much poorer (at least 27-fold worse) than those calculated using the back-calculated profiles, which suggests that

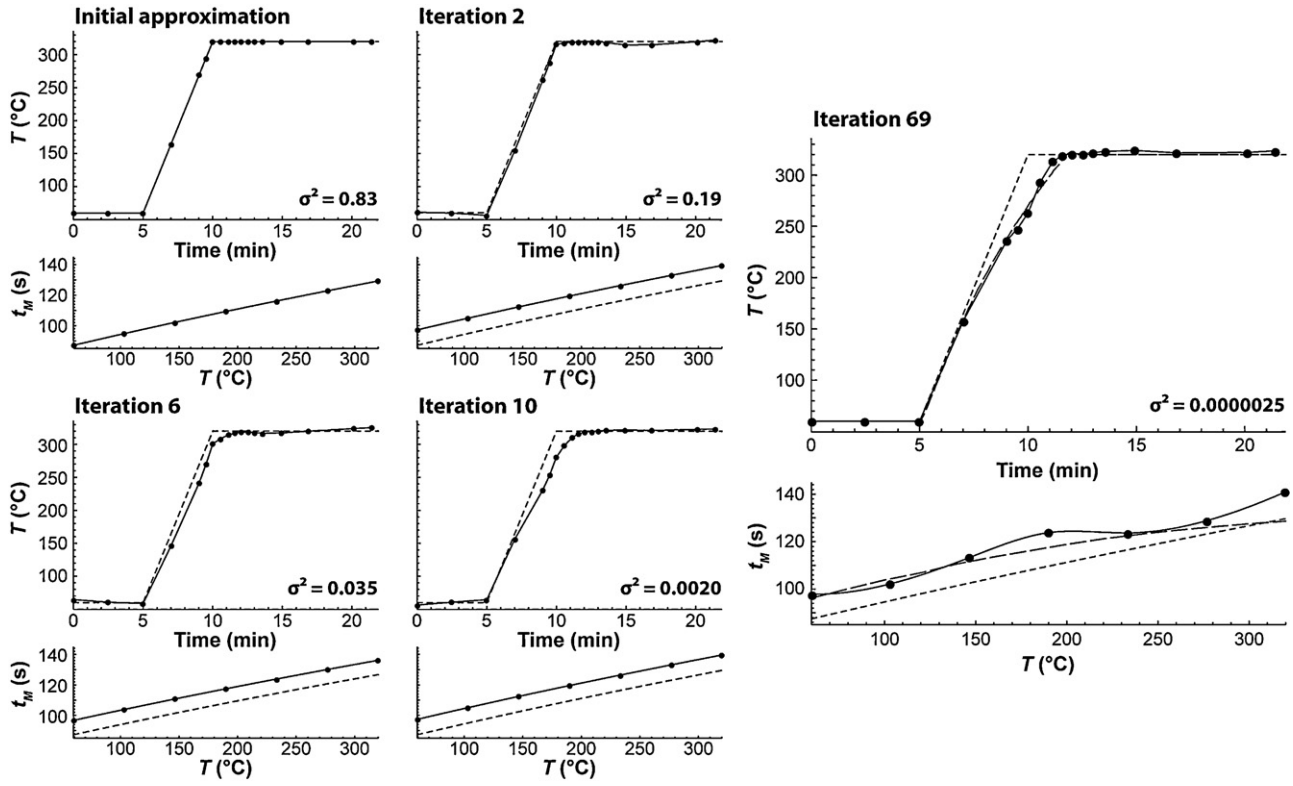

Fig. 5. Temperature and hold-up time profiles back-calculated for six different temperature programs. The dotted lines show the ideal profiles, the solid lines show the back-calculated profiles, and the dashed lines (only shown for the hold-up time profiles) show the measured hold-up time profiles. The temperature programs for non-linear ramps A–C are specified in Table 2 footnotes.

Table 2
Comparison of retention projection and linear retention indexing accuracy with different temperature programs, inlet pressures, flow rates, GC instruments, and stationary phase batches.

Run	GC instrument	Stationary phase batch	Ramp rate[b] (°C/min)	Inlet pressure[c] (kPa)	Flow rate (mL/min)	Standard deviation of prediction error (s)[a]			
						Retention projection		Linear retention indexing	
						w/back-calculated profiles	w/ideal profiles	Using LRIs calculated from run. . .	. . .To predict in this run
Part A: different temperature programs									
1	HP	A	52	50		±0.36	±32		
2	HP	A	26	50		±0.44	±19	**1**	±2.3
3	HP	A	13	50		±0.66	±22	**1**	±8.8
4	HP	A	6.5	50		±1.0	±30	**1**	±25
5	HP	A	Nonlinear A[d]	50		±1.0	±27	**1**	±30
6	HP	A	Nonlinear B[e]	50		±0.89	±28	**1**	±19
7	HP	A	Nonlinear C[f]	50		±0.87	±30	**1**	±25
Part B: different GC instruments									
1	HP	A	52	50		±0.36	±32		
8	Thermo	A	52	50		±0.66	±21	**1**	±1.1
9	Agilent	A	52	50		±0.57	±21	**1**	±0.69
2	HP	A	26	50		±0.44	±19		
10	Thermo	A	26	50		±0.78	±25	**2**	±1.1
11	Agilent	A	26	50		±0.46	±24	**2**	±0.64
3	HP	A	13	50		±0.66	±22		
12	Thermo	A	13	50		±0.88	±37	**3**	±1.1
13	Agilent	A	13	50		±0.62	±33	**3**	±0.56
4	HP	A	6.5	50		±1.0	±30		
14	Thermo	A	6.5	50		±1.7	±62	**4**	±1.1
15	Agilent	A	6.5	50		±1.1	±50	**4**	±0.60
Part C: different inlet pressures									
3	HP	A	13	50		±0.66	±22		
16	HP	A	13	100		±0.82	±19	**3**	±2.9
17	HP	A	13	150		±1.2	±18	**3**	±4.1
Part D: different flow rates									
20	Thermo	A	13		1	±0.84	±29		
22	Thermo	A	13		2	±0.91	±30	**20**	±3.3
23	Thermo	A	13		3	±1.2	±31	**20**	±3.6
Part E: different modes of flow regulation									
8	Thermo	A	52	50		±0.66	±21		
18	Thermo	A	52		1	±0.55	±15	**8**	±0.76
10	Thermo	A	26	50		±0.78	±25		
19	Thermo	A	26		1	±0.61	±19	**10**	±1.1
12	Thermo	A	13	50		±0.88	±37		
20	Thermo	A	13		1	±0.84	±29	**12**	±1.9
14	Thermo	A	6.5	50		±1.7	±62		
21	Thermo	A	6.5		1	±1.7	±51	**14**	±2.8
Part F: different stationary phase batches									
1	HP	A	52	50		±0.36	±32		
24	HP	B	52	50		±0.37	±37		
28	HP	C	52	50		±0.49	±33		
2	HP	A	26	50		±0.44	±19		
25	HP	B	26	50		±0.41	±28		
29	HP	C	26	50		±0.40	±25		
3	HP	A	13	50		±0.66	±22		
26	HP	B	13	50		±0.55	±35		
30	HP	C	13	50		±0.58	±30		
4	HP	A	6.5	50		±1.0	±30		
27	HP	B	6.5	50		±0.89	±49		
31	HP	C	6.5	50		±0.88	±42		

[a] Standard deviation in predicted retention times from experimental retention times for all solutes.
[b] 5 min at 60 °C, ramp to 320 °C at the specified ramp rate, hold 15 min.
[c] Relative to atmospheric pressure.
[d] 5 min at 60 °C, 26 °C/min to 190 °C, hold 15 min, 26 °C/min to 320 °C, hold 15 min.
[e] 5 min at 60 °C, 6.5 °C/min to 190 °C, 26 °C/min to 320 °C, hold 15 min.
[f] 5 min at 60 °C, 26 °C/min to 150 °C, hold 10 min, 26 °C/min to 235 °C, hold 10 min, 26 °C/min to 320 °C, hold 15 min.

the back-calculated profiles truly do provide an effective way to "calibrate out" non-idealities in the GC instrumentation.

To test the accuracy of linear retention indexing as the temperature program changes, we calculated LRIs for each of the 63 compounds in the 52 °C/min ramp (run **1**) and used them to predict retention times in the other temperature programs (runs **2**–**7**). For example, when LRIs measured in the 52 °C/min ramp (run **1**) were used to predict retention in the 26 °C/min ramp

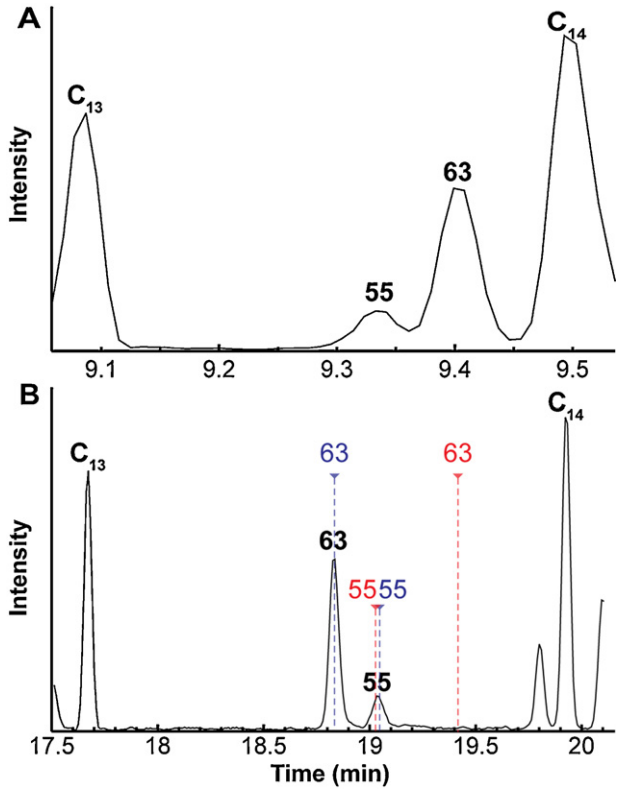

Fig. 6. Chromatogram A shows a short section of the 52 °C/min ramp (run **1**) with the peaks from the bracketing alkanes and compounds 55 and 63 labeled. Chromatogram B shows the same compounds in a 6.5 °C/min ramp. The dashed blue lines show *projected* retention times for the two compounds in the 6.5 °C/min ramp while the dashed red lines show retention times predicted using retention *indices* measured in the 52 °C/min ramp. Retention projection can account for changes in peak elution order, but retention indexing cannot. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

(run **2**), they were accurate to ±2.3 s. In that case, reducing the ramp rate by just twofold reduced the accuracy of retention indexing to fivefold lower than that of retention projections in the same ramp. *When the same 52 °C/min retention indices were used to predict retention in the 6.5 °C/min ramp (run **4**), they were only accurate to ±25 s, 25-fold worse than retention projections in that ramp.* In some of the non-linear ramps, retention indexing fared even worse—in run **5**, retention indexing was only accurate to ±30 s, 30-fold less accurate than the retention projections. We point out that we used the 52 °C/min rate to put the maximum stress on the methodology. Use of a less extreme reference rate i.e. one closer to the experimental rate would produce smaller deviations.

A striking example of the importance of accounting for the isothermal k vs. T relationships of different compounds is shown in Fig. 6. Chromatogram A shows compounds 55 and 63 bracketed by the C_{13} and C_{14} n-alkane standards in the 52 °C/min ramp (run **1**). Chromatogram B shows the same compounds in the 6.5 °C/min ramp (run **4**). Stark differences in the isothermal k vs. T relationships of compounds 55 and 63 are enough to actually cause a change in the order of elution. While linear retention indexing predicts the same elution order in both ramps (resulting in a 35 s error in the prediction of compound 63), *retention projection not only correctly predicts a reversal of their elution order, it predicts each of their retention times with less than 2.1 s error.*

3.2.3. Retention projection with different makes/models of GC instruments

Table 2, Part B shows the accuracy of retention projections on three different GC instruments, including an HP 5890 Series II (21 years old), a Thermo Trace GC Ultra (1 year old), and an Agilent 7890A (3 years old). The accuracy remained high on all three of them, though it was slightly worse on the Thermo GC. The higher error on that instrument could be related to its inlet and liner geometry, which are slightly different than the HP/Agilent models. It might also be a result of different MS transfer line lengths; in the HP system, the length of column in the transfer line region was 34 cm long and in the Agilent system it was about 32 cm long, but in the Thermo system it was about 57 cm long. Finally, it could be the result of a delay in the start of the retention time clock after autosampler injection. If the clock started significantly earlier or later on the Thermo GC, it could reduce the accuracy of retention projections. We plan to investigate these possibilities in future work.

To compare the accuracy of the retention projections to that of retention indexing, we determined retention indices from runs **1**–**4** on the HP instrument and used them to determine retention times in the same ramps on the Thermo and Agilent instruments. In faster ramps (the 52 °C/min and 26 °C/min ramps), the average error in retention projections was 30% lower than retention indices. On the other hand, in the slowest ramp (the 6.5 °C/min ramp), the retention projections showed an average of 69% more error than retention indices. Evidently, retention projections are more accurate than retention indexing on different GC instruments (at least on those used in this study) with relatively fast ramp rates (≥26 °C/min), while retention indexing is more accurate than retention projections with relatively slow ramp rates (≤6.5 °C/min), so long as *all* other experimental conditions are held constant.

3.2.4. Retention projection with different inlet pressures

Table 2, Part C shows the accuracy of retention projections with different inlet pressures in a 13 °C/min ramp. The accuracy of retention projections remained ≤±1.2 s, but when retention indices measured at 50 kPa (run **3**) were used to predict retention times at 100 kPa (run **16**) and 150 kPa (run **17**), their accuracy was poorer. In the 150 kPa run, they were only accurate to ±4.1 s, 3.4-fold less accurate than retention projections in the same run.

3.2.5. Retention projection with different flow rates

Likewise, Table 2, Part D shows the accuracy of retention projections with different flow rates. While the accuracy of retention projections remained ≤±1.2 s, when retention indices measured at 1 mL/min (run **20**) were used to predict retention times at 2 mL/min (run **22**) and 3 mL/min (run **23**), their accuracy was also poorer. In the 3 mL/min run, they were only accurate to ±3.6 s, threefold less accurate than the retention projections.

3.2.6. Retention projection with different modes of flow regulation

Retention projection should also be able to properly account for different modes of gas flow regulation (constant inlet pressure vs. constant flow rate), which affect the shape of the hold-up time profile. When retention indices measured at an inlet pressure of 50 kPa (runs **8**, **10**, **12**, and **14**) were used to predict retention times at a flow rate of 1 mL/min (runs **18**–**21**), they averaged 77% more error than retention projections (see Table 2, Part E). Meanwhile, the accuracy of retention projections was almost completely unaffected by a change in the mode of flow regulation.

3.2.7. Retention projection with different batches of stationary phase

If the selectivity of a column is not the same as the selectivity of the column used here to measure the isothermal k vs. T relationships, retention projections will not be accurate. Therefore, users currently need to use the same make/model of column we used (DB-5MS UI, 0.25 mm inner diameter, 0.25 μm film thickness) to ensure the correct selectivity. However, even when the same make/model of column is used, the accuracy of retention projections might lessen when different batches of the same phase are employed. To see if this is a problem, we tested the accuracy of retention projections on three DB-5MS UI columns, each containing stationary phase from a different batch. Table 2, Part F shows a comparison of batch A (the batch used to measure the isothermal retention data), batch B, and batch C. Evidently there is no significant loss of accuracy, suggesting that retention projections would be just as accurate as they were in this work with any new DB-5MS UI column.

4. Conclusions

The new retention projection system described in this work is both easy to use and accurate (to ±0.4 s) under a wide range of experimental conditions. We projected the retention times of a chemically diverse set of 63 compounds with different temperature ramps, different inlet pressures, different flow rates, different makes/models of GC instruments, and with different batches of stationary phase, *all with no worse than ±1.7 s accuracy*, though error was usually less than ±1 s, even in temperature ramps 40 min long. Since retention projection properly accounts for differences in the retention behavior of different compounds, *it can even account for changes in elution order that result from a change in the temperature program*. In contrast, changes in the temperature program, the inlet pressure, the flow rate, or the mode of gas flow regulation caused the accuracy of retention indices to drop, in most cases well below that of retention projections.

The new back-calculation algorithm makes it possible to measure the instrument's effective temperature and hold-up time profiles using only the retention times of a set of *n*-alkanes spiked into the sample which is subjected to programmed temperature elution. Using the back-calculated profiles to project retention times not only improved the accuracy of retention projections (compared to when the profiles were assumed to be ideal), it also makes the system exceedingly easy to use. From a user's perspective, it requires no more effort than retention indexing.

We developed a public website and open-source software at http://www.retentionprediction.org/gc to demonstrate the new GC retention projection system. Currently there are only 63 compounds in the initial isothermal retention database, but we plan to greatly expand it in the future. We also plan to test the robustness of the system under circumstances faced by a typical user (high/low sample loadings, diverse sample matrices, repeated injections of messy samples, etc.), and to develop a new type of system suitability check that uses the back-calculated profiles to automatically detect instrument and/or column problems. We encourage interested readers to try out the software and email us with comments and suggestions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2012.09.048.

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