Reliability of Van den Dool Retention Indices in the Analysis of Essential Oils

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Abstract

The aim of this study is to evaluate how extensive retention indices (RIs) can be identifying an essential oil component within a single laboratory. The essential oils of *Matricaria chamomilla* L., *Tagetes lucida* Cav., and *Artemisia roxburghiana* Wall. are investigated with different columns and under different analytical conditions. These oils are analyzed with a GC unit and columns coated with the same stationary phases (polysiloxane OV-1 and polyethylene glycol CW-20M) but different characteristics (producer and inner diameter) under different analytical conditions (temperature program and mobile phase conditions). The great number of data obtained are used to determine what parameters other than stationary phase influence RI reliability, the range of variation of RIs with different columns or under different operating conditions, and the validity of D-RI measurements.

Introduction

One of the main goals of research in gas chromatography (GC) is to make the chromatographic data dependent on only the chromatographic phenomenon (i.e., the three-term interaction analyte–mobile phase–stationary phase). Because the mobile phase should have less influence, the chromatographic retention of the analyte should depend only on the stationary phase and be as independent as possible from operating conditions. This would make the chromatographic data (specifically retention) reliable and usable in identification at an interlaboratory level.

In introducing retention indices (RIs), first Kováts (1) and then Van den Dool and Kratz (2) aimed to make retention values independent from operating conditions. They achieved this by measuring retention relative to a homologous series of hydrocarbons.

Retention indices are fundamental in making retention data comparable, although many problems still exist: stationary phase performance varies as a function of temperature; mobile phase characteristics depend on temperature in temperature-programmed analysis; instrumental control (oven temperature and mobile phase pneumatic control in particular) is limited; and software has no RI option (if available at all, the RI option is not a standard part of even the most recent GC elaboration software).

In addition to this, GC operators apparently prefer to reason in terms of retention time rather than in terms of chromatographic behavior, although this often causes serious error. This attitude can be justified with pure compounds or very simple mixtures, but not with complex mixtures such as essential oils.

The aim of this paper was to evaluate the reliability of Van den Dool RIs when applied to the GC analysis of the essential oils of *Matricaria chamomilla* L., *Tagetes lucida* Cav., and *Artemisia roxburghiana* Wall. These oils were analyzed with several columns coated with various stationary phases: 2 different stationary phases, the same stationary phase but with columns supplied by different manufacturers, and the same stationary phase but with columns having different inner diameters. The oils were also analyzed under different conditions: constant pressure, constant flow, constant average linear carrier gas velocity, and

Table I. List of the Columns Used in This Investigation

Peak	Length (m)	Inner diameter (mm)	df (µm)	Origin	Column ID
OV-1 (methylpolysiloxane)					
1	25	0.18	0.3	HM*	A
2	25	0.25	0.3	HM*	B
3	25	0.32	0.3	HM*	C
4	25	0.32	0.3	CA†	D
CW-20M (polyethylenglycole)					
5	25	0.25	0.25	HM*	E
6	25	0.32	0.25	HM*	F
7	25	0.32	0.25	CA†	G

* HM, lab-made.
† CA, commercially available.

different starting temperatures and rates. The RIs of a group of selected test compounds in each oil were compared. This paper will not describe the evolution of RIs in detail; their theory and practice was discussed in depth 30 years after their introduction by Kováts (3).

Table II. RIs of the Selected Components for Each of the 3 Essential Oils*

Peak	Compound	RI	Peak	Compound	RI
Matricaria chamomilla L.			*Artemisia roxburghiana* Wall.		
1	*trans*-β-Farnesene	1442	1	Santolina triene	901
2	Bisabolol oxide B	1619	2	α-Pinene	923
3	α-Bisabolone oxide A	1637	3	Camphene	934
4	α-Bisabolol	1649	4	Sabinene	957
5	Chamazulene	1674	5	β-Pinene	960
6	Bisabolol oxide A	1702	6	1,8-Cineole	1012
7	Spiro-ether	1805	7	γ-Terpinene	1042
			8	Artemisia alcohol	1065
Tagetes lucida Cav.			9	α-Thujone	1076
1	Myrcene	979	10	β-Thujone	1086
2	*trans*-β-Ocimene	1034	11	Camphor	1107
3	Linalol	1080	12	Sabinol	1114
4	Estragole	1168	13	Borneol	1136
5	Anethole	1251	14	Artemisia acetate	1150
6	Methyl-eugenol	1367	15	α-Terpineol	1161
7	β-Caryophyllene	1396	16	Bornyl acetate	1257
8	Germacrene D	1453	17	*trans*-Sabinyl acetate	1265
			18	β-caryophyllene	1395
			19	Germacrene D	1454
			20	Bicyclogermacrene	1469

* The conditions were as follows: column, OV-1(B); injection, split; split ratio, 1:20; temperature, 230°C; detector, FID; detector temperature, 250°C; temperature program, 50°C (1 min) to 220°C (10 min) at 3°C/min; carrier gas, hydrogen; constant flow, 1.5 mL/min.

Table III. RIs of the Selected Components for Each of the 3 Essential Oils*

Peak	Compound	RI	Peak	Compound	RI
Matricaria chamomilla L.			*Artemisia roxburghiana* Wall.		
1	*trans*-β-Farnesene	1658	1	Santolina triene	—
2	Bisabolol oxide B	2099	2	α-Pinene	1018
3	α-Bisabolone oxide A	2132	3	Camphene	1059
4	β-Bisabolol	2201	4	Sabinene	1116
5	Chamazulene	2334	5	β-Pinene	1104
6	Bisabolol oxide A	2338	6	1,8-Cineole	1201
7	Spiro-ether	—	7	γ-Terpinene	1236
			8	Artemisia alcohol	1510
Tagetes lucida Cav.			9	α-Thujone	1404
1	Myrcene	1159	10	β-Thujone	1422
2	*trans*-β-Ocimene	1247	11	Camphor	1488
3	Linalool	1553	12	Sabinol	1689
4	Estragole	1656	13	Borneol	1695
5	Anethole	1807	14	Artemisia acetate	1419
6	Methyl-eugenol	2006	15	α-Terpineol	—
7	β-Caryophyllene	1566	16	Bornyl acetate	1560
8	Germacrene D	1675	17	*trans*-Sabinyl acetate	1637
			18	β-caryophyllene	1566
			19	Germacrene D	1675
			20	Bicyclogermacrene	1698

* The conditions were as follows: column, CW-20M(E); injection, split; split ratio, 1:20; temperature, 230°C; detector, FID; detector temperature, 250°C; temperature program, 50°C (1 min) to 200°C (10 min) at 3°C/min; carrier gas, hydrogen; constant flow, 1.5 mL/min.

Experimental

Essential oils

The essential oils of *M. chamomilla*, *T. lucida*, and *A. roxburghiana* were obtained by hydrodistillation using a modified Clevenger apparatus (4).

GC analysis

GC analyses were carried out on a Hewlett-Packard (Milan, Italy) HP 6890 GC unit with mobile phase electronic pressure control and fitted with an automatic HP 6890 series injector. Hydrogen was used as the carrier gas. Split injection was made at a split ratio of 1:20 and a temperature of 230°C. The flame-ionization detector (FID) temperature was 250°C.

Table I lists the columns used for the experiments: methylpolysiloxane (OV-1) and polyethylene glycol (Carbowax CW-20M). Columns A, B, C, E, and F were prepared in the laboratory, and columns D and G were supplied by Hewlett-Packard.

Analytical conditions

GC analysis conditions are given in the legends of each table and figure. Each analysis was repeated 3 times.

RI determination

A C_8–C_{25} hydrocarbon standard mixture was analyzed under the same operating conditions as the essential oils. The RIs were calculated according to the RI Van den Dool and Kratz equation (2). The chromatographic data from the detectors were processed with Asterix data treatment software (Hewlett-Packard, Avondale, PA) on an HP Vectra VL 5/100 personal computer (Hewlett-Packard, Grenoble, France). The RIs were calculated with an improved version of a lab-made program described elsewhere (5).

Results and Discussion

The results reported here are part of the authors' everyday work on the latest generation of GC instruments; they comprise more than 1300 GC injections carried out on the essential oils of *M. chamomilla*, *T. lucida*, and *A. roxburghiana* over a 4-month period. Tables II and III list the components for each essential oil considered in this investigation with their Van den Dool RIs deter-

mined on OV-1(B) and CW-20M(E) columns. These data were also taken as reference data. Figures 1–3 report the CGC patterns of the three essential oils under investigation analyzed by both OV-1(B) and CW-20M(E) columns.

The RIs were calculated from the Van den Dool equation; a broken-line function was adopted, because it gave more uniform results in general than the other functions (linear and polynomial). Van den Dool indices were calculated from the component elution temperatures automatically obtained from the retention times. A hydrocarbon homologous series was used as a reference for both apolar and polar stationary phases, although a homologous series of fatty acid ethyl esters would have given better results with polar columns (6).

In this study, an identification tolerance of ± 3 RI units was chosen. Some authors claim to have obtained results accurate to the first decimal place, and that under undefined analytical conditions (in terms of temperature program in particular), it is easy to work to limits of ± 2 RI units. However, in the present authors' experience, such precision is only possible under well-defined analysis conditions. The RIs are reported here as whole numbers; RI differences are only reported to the first decimal place to facilitate the evaluation of the results. Moreover, RIs were calculated on analyte amounts within the column capacity, because the RI value of an analyte varies when the column is overloaded (7).

The development of GC instruments with electronic pressure control of the mobile phase and GC ovens in which temperature is strictly controlled and evenly distributed has overcome several problems with instrumentation and gives rigorous control of the mobile phase parameters (flow rate, pressure, and average linear velocity) and thermal parameters over the entire GC run. Thus, the chromatographic process (and hence, retention) becomes highly reproducible.

In recent work in the present authors' laboratory, under fixed conditions, an RI precision of 1 unit was maintained over 1 month (Table IV) for the essential oil components of both *Tagetes lucida* on an OV-1(B) column and *Matricaria chamomilla* on a CW-20M(E) column. These RIs were calculated referring to a hydrocarbon set analyzed the same day as the samples. When RIs were referred to the same hydrocarbon set over the whole month, RI differences were outside the fixed limits, thus confirming that the hydrocarbon reference set must be periodically renewed (e.g., every 2–3 days).

The very good reproducibility of these results does not mean

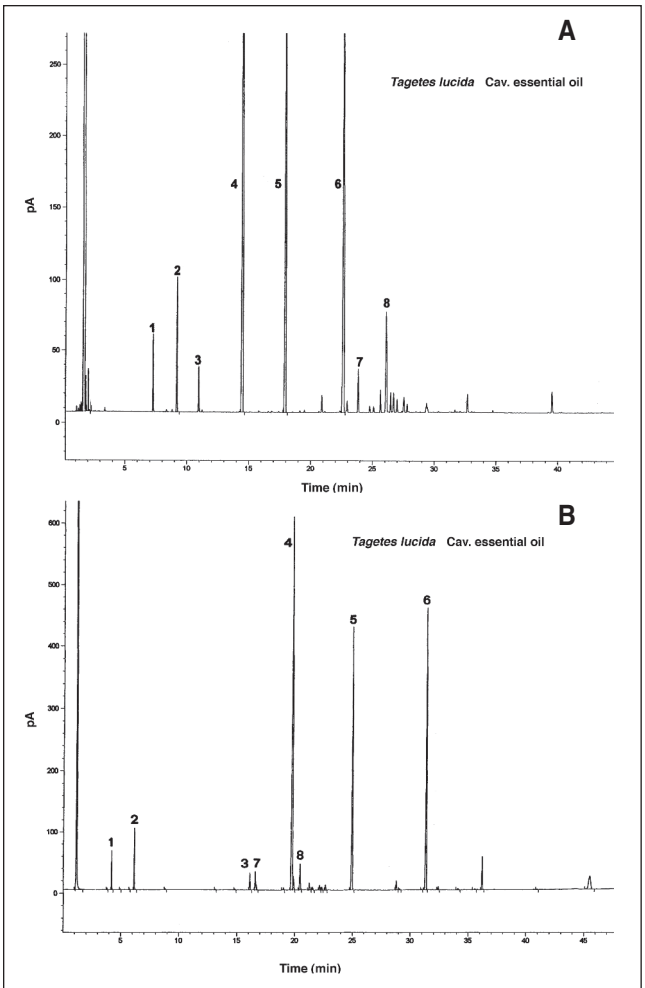

Figure 1. CGC patterns of *Tagetes lucida* Cav. essential oil: OV-1 column B with a temperature program from 50°C (1 min) to 220°C (10 min) at 3°C/min (A), CW-20M column E with a temperature program from 50°C (1 min) to 200°C (10 min) at 3°C/min (B). GC conditions: injection, split; split ratio, 1:20; temperature, 230°C; detector, FID; detector temperature, 250°C; carrier gas, hydrogen; constant flow, 1.5 mL/min.

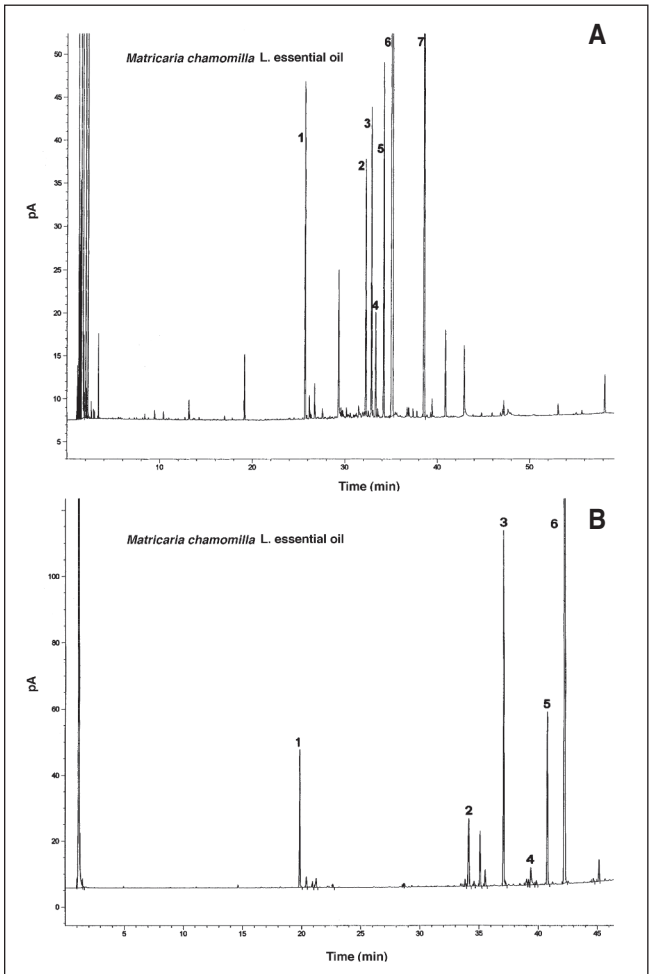

Figure 2. CGC patterns of *Matricaria chamomilla* L. essential oil: OV-1 column B (A) and CW-20M column E (B). GC conditions were the same as in Figure 1.

that RIs do not vary when different analysis conditions are applied, nor does it indicate to what extent GC data from different GC systems or laboratories are comparable.

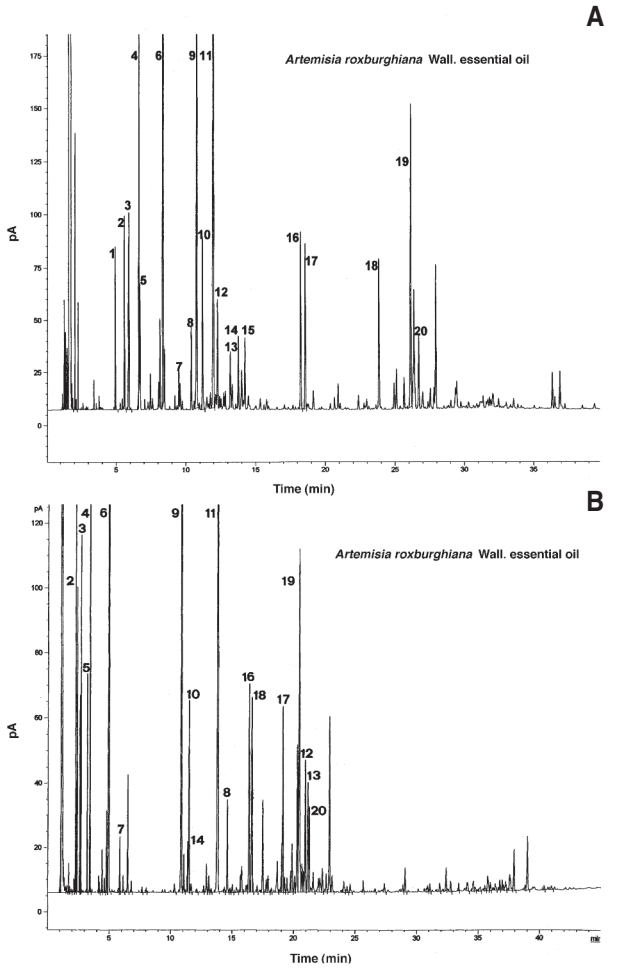

Figure 3. CGC patterns of *Artemisia roxburghiana* Wall. essential oil: OV-1 column B (A) and CW-20M column E (B). GC conditions were the same as in Figure 1.

This study examined the influence of the GC parameters on the reproducibility of RIs when analyses are carried out on the same GC unit. The parameters that must be investigated are those that should be reported in a scientific publication to characterize a GC analysis: mobile phase dynamic conditions, stationary phase, film thickness, column length, column inner diameter, initial temperature and rate, and column origin. This study did not investigate film thickness or column length. Stationary phase film thickness was not investigated because, in general, it influences the chromatographic interaction, affecting the elution temperature of an analyte and, as a consequence, its retention (8). Column length was not investigated because between 15 and 30 m, it has little or no influence on RIs, although it does influence separation.

As a first step, the influence of mobile phase dynamic conditions on RIs when all instrumental parameters are optimized was investigated. Table V reports the RIs of *T. lucida* essential oil components analyzed under constant pressure, constant flow, and constant average linear velocity conditions with OV-1(B) and CW-20M(E) columns. This series of analyses showed that, under the same temperature conditions, the mobile phase dynamic conditions only slightly influence RIs on both OV-1 and CW-20M columns.

The RIs differed when different starting temperatures and rates were applied. Table VI reports the RI variations as a function of starting temperature and rate for chamomile oil components on both OV-1(B) and CW-20M(E) columns. From these results, it appears that (1) with both columns, the results with different programme rates are outside the fixed tolerance limits; (2) the RI value of each compound varied nonuniformly with the different program rates; and (3) different starting temperatures with the same program rate have much less influence on the RI differences.

Therefore, program rate is the factor mainly responsible for RI variation, and RIs may only be compared to those of another laboratory when temperature conditions are specified.

The differences in RIs might partly be explained by the variation of polarity of a stationary phase as a function of temperature, a factor whose importance is not always fully recognized. Grob and Grob (9) studied this problem in depth and showed that for the majority of solute/stationary phase combinations, an increase in temperature results in an increase in polarity. As a consequence, a variation in an analyte elution temperature (due to a different temperature program rate, etc.) involves a variation in its RI. This phenomenon is emphasized by the increasing degree of control that sophisticated technology offers (electronic pressure and thermal control) and the use of very-high-efficiency capillary columns.

The next parameter is column diameter. The 3 essential oils were analyzed on 3 OV-1 columns (columns A–C) and 2 CW-20M columns (columns E and F) with a series of inner diameters. Table VII compares the RI variations of *A. roxburghiana* oil components when analyzed with columns of different diameter under optimal average linear velocity. With the OV-1 columns, the values fit very well with the fixed tolerances, the largest

Table IV. Reproducibility Over Time of RIs of *Matricaria chamomilla* L. Essential Oil Component on OV-1(B) and *Tagetes lucida* Cav. Essential Oil Components on CW-20M(E)*

Peak	Compound	RI'	RI''	Peak	Compound	RI'	RI''
Matricaria chamomilla L.				*Tagetes lucida* Cav.			
1	*trans*-β-Farnesene	1442	1441	1	Myrcene	1159	1157
2	Bisabolol oxide B	1619	1618	2	*trans*-β-Ocimene	1247	1246
3	α-Bisabolone oxide A	1637	1637	3	Linalol	1553	1553
4	α-Bisabolol	1649	1649	4	Estragole	1656	1656
5	Chamazulene	1674	1674	5	Anethole	1807	1805
6	Bisabolol oxide A	1702	1701	6	Methyl-eugenol	2006	2005
7	Spiro-ether	1805	1805	7	β-Caryophyllene	1566	1566
				8	Germacrene D	1675	1673

* GC conditions were as follows: injection, split; split ratio, 1:20; temperature, 230°C; detector, FID, detector temperature, 250°C; temperature program, 50°C (1 min) to 220°C (10 min) at 3°C/min; carrier gas, hydrogen; constant flow, 1.5 mL/min.
† Reference RIs.
‡ RIs calculated 1 month later under the same conditions.

deviations being within a range of 2–3 RI units on average. When 0.25- and 0.32-mm CW-20M columns are compared, the RI differences increase; the smallest difference is a-pinene (2.6 units), whereas the biggest is borneol (19.4 units). There is also an inversion in retention of sabinol and borneol, although the stationary phase, surface deactivation process, and fused silica origin were the same. These results indicate that with OV-1 as a stationary phase, the column's inner diameter influences the RI deviation within an acceptable range, but for CW-20M columns, the inner diameter must be specified.

Table V. RIs of *Tagetes lucida* Cav. Essential Oil Components Under Different Mobile Phase Conditions*

Peak	Compound	Constant pressure (7.7 psi)	Constant flow (1.5 mL/min)	Constant average linear velocity (44 cm/s)
OV-1(B)				
1	Myrcene	979	979	979
2	*trans*-β-Ocimene	1034	1034	1034
3	Linalol	1080	1080	1080
4	Estragole	1168	1168	1168
5	Anethole	1252	1251	1252
6	Methyl-eugenol	1367	1367	1367
7	β-Caryophyllene	1397	1396	1397
8	Germacrene D	1453	1453	1454
CW-20M(E)				
1	Myrcene	1159	1159	1159
2	*trans*-β-Ocimene	1246	1247	1246
3	Linalol	1552	1553	1552
4	Estragole	1657	1656	1656
5	Anethole	1809	1807	1807
6	Methyl-eugenol	2007	2006	2006
7	β-Caryophyllene	1567	1566	1566
8	Germacrene D	1677	1675	1676

* GC conditions were as follows: injection, split; split ratio, 1:20; temperature, 230°C; detector, FID, detector temperature, 250°C; temperature program, 50°C (1 min) to 200°C (10 min) at 3°C/min; carrier gas, hydrogen.

Table VI. RI Differences in *Matricaria chamomilla* L. Essential Oil Components Under Different Initial Temperatures and Rates

		RI	RI variations			
Peak	Compound	IT 50, TR 3	IT 50, TR 1.5–3	IT 50, TR 1.5–6	T 50, ITR 3–6	IT 100, TR 3–3
OV-1(B)						
1	*trans*-β-Farnesene	1442	2.0	3.6	1.5	0.4
2	Bisabolol oxide B	1619	8.1	16.9	8.8	4.4
3	α-Bisabolone oxide A	1637	9.0	18.6	9.6	4.3
4	α-Bisabolol	1649	6.1	13.3	7.2	3.6
5	Chamazulene	1674	11.6	24.7	13.1	5.4
6	Bisabolol oxide A	1702	9.4	19.0	9.7	3.1
CW-20M(E)						
1	*trans*-β-Farnesene	1658	3.5	6.5	3.0	6.1
2	Bisabolol oxide B	2099	14.7	30.2	15.5	6.5
3	α-Bisabolone oxide A	2132	18.5	37.2	18.7	6.5
4	α-Bisabolol	2201	7.4	14.6	7.3	2.5
5	Chamazulene	2334	25.5	51.4	26.0	3.7
6	Bisabolol oxide A	2388	14.4	29.0	14.6	1.6

* GC conditions were as follows: injection, split; split ratio, 1:20; temperature, 230°C; detector, FID, detector temperature, 250°C; carrier gas, hydrogen; constant flow, 1.5 mL/min.
† IT is the initial temperature expressed in °C. TR is the temperature rate expressed in °C/min.

The next source of deviation of RIs is column origin. Although it is relatively easy to find polysiloxane columns from different producers with comparable performances, it is difficult to obtain comparable retentions and elution orders with polyethylene glycol columns from different producers. This is probably because each column manufacturer makes a different polyethylene glycol column available; CW-20M is an acronym pertaining to polyethylene glycols with different structures or different polymerization or different polymers that have been applied differently to the inner walls of the column (CW-20M polymer directly coated, prepolymer polymerized on the column walls directly, autocrosslinkable polymer or prepolymer, etc.). The results reported here are only partial because of the statistically insignificant number of columns tested, but they give an idea of the RI deviations involved. An OV-1(C) and a CW-20M(F) column prepared in the laboratory were compared with 2 corresponding commercially-available columns, OV-1(D) and CW-20M(G). Table VIII reports the results obtained with *M. chamomilla* essential oil components. With the OV-1 columns, RI deviations varied from 1 unit for *trans*-β-farnesene to 9 units for chamazulene. With the polyethylene glycol columns, the difference was much higher, because RI deviations varied from 20 units for *trans*-β-farnesene to 65 units for chamazulene. Moreover, for both stationary phases, commercial columns had slightly higher retention in all cases. Although the number of columns tested was very small, the OV-1 column's origin influenced the RI deviation within an acceptable range, whereas with polyethylene glycol, the range was too wide, so column origin must be stated.

Identifications through RIs are generally only considered significant when 2 successful matchings are obtained from different-polarity stationary phases. When a suitable reference RI data base is available, the percentage of correct identifications obtainable through RIs is approximately 65% with 1 stationary phase, approximately 80% with 2 different-polarity columns, and above 90% with 3 columns. The latter percentages are close to that obtainable with mass spectrometry (generally approximately 90%). Because a GC system allowing simultaneous injection into 2 columns is very easy to assemble and today's processing systems can easily handle 2 detector signals, a manual or automatic cross-identification proce-

Table VII. RI Differences in *Artemisia roxburghiana* Wall. Essential Oil Components with Columns with Different Inner Diameters*

Peak	Compound	OV-1 columns				CW-20M columns	
		RI (0.25 mm)	RI variations			RI (0.25 mm)	RI variations (0.25–0.32 mm)
			0.18–0.32 mm	0.25–0.32 mm	0.18–0.25 mm		
1	Santolina triene	901	1.3	0.3	1.0	—	—
2	α-Pinene	923	1.2	0.4	0.9	1017	2.6
3	Camphene	933	1.3	0.4	0.9	1058	4.2
4	Sabinene	957	1.6	0.3	1.3	1116	4.1
5	β-Pinene	959	1.3	0.3	1.1	1103	3.3
6	1,8-Pineole	1011	2.2	–0.7	2.8	1201	6.4
7	γ-Terpinene	1041	1.0	0.3	0.7	1235	5.6
8	Artemisia alcohol	1065	2.3	–0.3	2.6	1510	11.2
9	α-Thujone	1075	2.3	–0.9	3.1	1404	9.2
10	β-Thujone	1086	1.6	–0.8	2.4	1421	8.9
11	Camphor	1107	2.2	–1.2	3.3	1488	10.4
12	Sabinol	1114	1.7	–1.0	2.7	1689	7.9
13	Borneol	1136	1.2	–0.8	2.0	1695	19.4
14	Artemisia acetate	1150	1.4	–0.3	1.6	—	—
15	α-Terpineol	1161	1.4	–0.7	2.1	—	—
16	Bornyl acetate	1258	1.6	–0.6	2.2	1560	10.1
17	*trans*-Sabinyl acetate	1265	1.6	–0.9	2.5	1637	10.6
18	β-Caryophyllene	1396	1.4	1.0	0.4	1566	9.1
19	Germacrene D	1455	1.8	0.5	1.3	1676	10.8
20	Bicyclogermacrene	1470	1.4	0.6	0.8	1699	10.8

* GC conditions were as follows: injection, split; split ratio, 1:20; temperature, 230°C; detector, FID, detector temperature, 250°C; temperature program, 50°C (1 min) to 200°C (10 min) at 3°C/min; carrier gas, hydrogen; constant flow, 1.5 mL/min.; constant average linear velocity, optimized for each inner diameter.

Table VIII. RI Variations in *Matricaria chamomilla* L. Essential Oil Components with Columns from Different Origins Under Different Dynamic Mobile Phase Conditions*

Peak	Compound	RI variations			
		RI at constant flow (2.3 mL/min)	Constant pressure (4.7 psi)	Constant flow (2.3 mL/min)	Constant average linear velocity (44 cm/s)
OV-1, 25 m × 0.32-mm i.d., 0.3-µm df					
1	*trans*-β-Farnesene	1443	–1	–1	–1
2	Bisabolol oxide B	1616	–7	–6	–6
3	α-Bisabolone oxide A	1633	–8	–7	–7
4	α-Bisabolol	1649	–5	–4	–4
5	Chamazulene	1669	–9	–8	–8
6	Bisabolol oxide A	1698	–6	–5	–6
7	Spiro-ether	1804	–5	–4	–4
CW-20M, 25 m × 0.32-mm i.d., 0.25-µm df					
1	*trans*-β-Farnesene	1646	–20	–21	–20
2	Bisabolol oxide B	2073	–46	–45	–45
3	α-Bisabolone oxide A	2104	–51	–50	–49
4	α-Bisabolol	2180	–36	–36	–36
5	Chamazulene	2299	–65	–63	–63
6	Bisabolol oxide A	2359	–49	–47	–47

* GC conditions were as follows: injection, split; split ratio, 1:20; temperature, 230°C; detector, FID, detector temperature, 250°C; temperature program, 50°C (1 min) to 200°C (10 min) at 3°C/min; carrier gas, hydrogen.

dure is easy to develop (5). The dual-column GC system also makes it possible to determine Δ-RI. Δ-RI expresses the retention of an analyte on 2 columns in a single number that is the difference between the RIs of a single analyte on 2 columns coated with different-polarity stationary phases. Table IX reports Δ-RIs of 3 pairs of components of the 3 essential oils investigated obtained with 3 different pairs of columns: OV-1(B) and CW-20M(E) (lab-made), OV-1(C) and CW-20M(F) (lab-made), OV-1(D) and CW-20M(G) (commercially available). For each essential oil, the components showing the minimum and maximum Δ-RI differences on the 3 pairs of columns were chosen: *trans*-β-farnesene and α-bisabolol in chamomile oil, myrcene and methyl eugenol in *Tagetes lucida* oil, and α-pinene and sabinol in *Artemisia roxburghiana* oil. Δ-RI values for each pair of columns are highly reproducible, provided that the same temperature rate is applied. However, with the exception of a-pinene, the results of the 3 pairs of columns are not comparable. Δ-RIs are very useful when analysis conditions and column characteristics and origins are specified. For Δ-RI, the reliability of column performance is fundamental, and this is particularly true with polyethylene glycol phases, because when one of the 2

Table IX. D-RIs of Some Components of *Matricaria chamomilla* L., *Tagetes lucida* Cav., and *Artemisia roxburghiana* Wall. Essential Oils on 3 Different Pairs of Columns*

Compound	CW-20M(E) and OV-1(B)		CW-20M(F) and OV-1(C)		CW-20M(G) and OV-1(D)	
	OV-1 RI	Δ-RI	OV-1 RI	Δ-RI	OV-1 RI	Δ-RI
trans-β-Farnesene	1442	216	1443	205	1444	223
α-Bisabolol	1651	552	1651	533	1656	564
Myrcene	979	180	978	174	980	185
Methyl-eugenol	1367	641	1370	622	1370	654
α-Pinene	923	95	922	93	924	97
Sabinol	1114	576	1114	564	1117	591

* GC conditions were as follows: injection, split; split ratio, 1:20; temperature, 230°C; detector, FID, detector temperature, 250°C; temperature program, 50°C (1 min) to 200°C (10 min) at 3°C/min; carrier gas, hydrogen; constant pressure, optimized for each inner diameter.

columns has to be replaced, the new one must have the same performance. This restriction might be overcome with new software that has an option to normalize the new column versus the previous one, provided that the same stationary phase is used. The software automatically recalculates the analysis conditions for a reference sample, giving full matching chromatograms produced with the 2 columns (retention time locking).

Conclusion

Retention indices are fundamental in locating and identifying components in a complex mixture using GC. The latest generation of GC instruments with electronic pressure control of the mobile phase and GC ovens in which temperature is strictly controlled and evenly distributed allows the high reproducibility of RIs over time when the same analytical conditions are applied. Variations of RIs within or near the fixed tolerance limits (± 3 RI units) are obtained, even when polysiloxane columns with different inner diameters and of different origins are adopted. On the other hand, RI variations are outside the fixed tolerance limits with both polysiloxane and polyethylenglycol columns when the starting temperature and rate vary and when polyethylene glycol columns with different inner diameters and of different origins are adopted.

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