The reliability of Kovats Indices for identification

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Since 2013 the Volatile Compounds in Food (VCF) internet database has contained retention indices, using the most reliable values of correctly identified compounds from the literature. This boosts the reliability of the mean values. It is especially those values obtained for polar GC-column coatings that have to be handled with care.

Introduction

In 1963 the late Cor Weurman started the VCF database. Based on data published in the scientific literature, this is a compilation of naturally occurring volatile compounds in basic food products. The data are available by subscription via the internet (www.vcf-online.nl) [1].

Only reliable data from literature are used; the occurrences of tentatively identified compounds are not. Only when a compound is identified using two different techniques is the compound considered to be positively identified. In most cases, compounds are identified on the basis of their mass spectrum and retention time, the latter being compared, by preference, with those of authentic reference compounds. This comparison, however, may be rather time consuming and often the reference compounds are not readily available. Consequently, many researchers make use of retention indices published by other authors, and this generates a huge amount of RI values.

In 2013, at the 50th anniversary of the VCF database, a start was made on implementing Retention Indices (RI) data based on n-alkanes. The RI data for four types of column coatings have been collected and, for the sake of convenience, characterised as DB-1, DB-5, DB-17 and DB-Wax. At this moment, 21,500 RI values for 3,300 different volatile compounds have been compiled in the VCF database. From a certain reference all available RI values are added to the database, no matter how many values of a compound have already been collected. All values are shown together with the mean value.

Results and discussion

The RI values from different literature references for an apolar compound (like limonene on DB-5), varies between 1021–1042, with a mean value of 1030. For the same compound on a DB-Wax the RI range is 1177-1218 with a mean value of 1197.

For a more polar compound like linalool the range on a DB-5 is 1094-1105 and on a DB Wax 1522- 1566. In these examples, it hardly matters whether a compound is polar or apolar for the range of RI values.

In terms of all data, it can be concluded that the RI data on apolar coatings like a DB-1 and a DB-5 are much closer (variation approx. 20 units) than on polar coatings like DB-Wax (variation up to 100 units).

In the database a question mark (?) shows when the RI value for a compound on a certain coating seems to be unreliable. This is not done according to a strict rule but made depending on the range of RI values collected for a certain compound. Here the range of RI values for hexanoic acid is presented. The mean value is 1840.

All Kovats RI values for hexanoic acid on DB-Wax
1797 1807 1807 1813 1823 1823 1825 1829 1835 1836 1836 1840 1840 1843 1847 1850 1852 1854 1856 1860 1860 1862 1863 1863 1870 1871 1935?

The RI values are regularly spread over a range of almost 80 units. It was decided that the value of 1935 was excessive and it is also questionable whether the same applies for the value of 1797.

Only from a large number of RI values can it be determined which data come within an acceptable range and which exceed this range, as shown in the example for pyrrole (below). Since it is not (yet) clear from such a small set of data which value is excessive, the mean value has been selected as the most realistic value. When more values become available, the mean value can be made more reliable.

Column Type	Average RI	All Kovats RI values (pyrrole)
DB-Wax	1493	1460 1526

Another example is the range of RI values for methional on a polar coating. Although the range of RI for a DB-Wax coating is certainly quite narrow, the value 1414 is considered to be excessive, although this does not mean that when more RI values become available, these may fall between 1414 and 1440, which could imply that the value of 1414 is no longer excessive. This is typical of the way this database uses the data.

Column Type	Average RI	All Kovats RI values (methional)
DB-Wax	1453	1414? 1440 1443 1450 1455 1456 1458 1461 1464

Another way to check the reliability of the RI value is to look for the values obtained for the four coatings. The RI values for a certain compound increase as the polarity of the column coating increases, this is shown in Table 1.

Table 1. RI values for different column coatings

Compound	DB-1	DB-5	DB-17	DB Wax
Terpinolene	1079	1086	1112	1286
α-Terpineol	1173	1190	1299	1689
Nonanal	1085	1103	1190	1391
Hexanoic acid	957	998	1185	1840
Ethyl hexanoate	983	998	1054	1229
Trimethylpyrazine	978	1003	1079	1391
Methional	865	906	1044	1453

The database also enables a review of all the data from the reference literature for which a value has been obtained. The RI values from a literature reference are displayed with an indication of whether a value is lower or higher than the mean value. The indication ≈ differs for each column coating. For the coatings DB-1 and DB-5, a range of five units is used; for DB-17 ten units and for DB-Wax twenty units.

A typical example of how new RI values reported in a certain literature reference are compared against the existing data base is given in Table 2.

Table 2. Comparison of a new data source against the mean database values, see foot note for explanation

GC phase	RI value New and mean	Number of references	Compound
DB-5	1034≈ 1037	(n=18)	(Z)-β-ocimene
DB-5	1045≈ 1046	(n=17)	(E)-β-ocimene
DB-5	1058≈ 1059	(n=23)	γ-terpinene
DB-5	1069= 1069	(n=8)	cis-sabinene hydrate
DB-5	1086= 1086	(n=26)	terpinolene
DB-5	1089≈ 1091	(n=10)	2-nonanone
DB-5	1101≈ 1097	(n=3)	2-nonanol
DB-5	1099≈ 1098	(n=36)	linalool
DB-5	1102≈ 1100	(n=4)	3-methylbutyl 2-methylbutanoate
DB-5	1107≈ 1105	(n=3)	2-methylbutyl 3-methylbutanoate
DB-5	1119> 1114	(n=9)	β-thujone
DB-5	1127= 1127	(n=5)	α-campholene aldehyde
DB-5	1142≈ 1140	(n=4)	trans-pinocarveol
DB-5	1142≈ 1141	(n=3)	cis-β-terpineol
DB-5	1124? < 1141	(n=4)	trans-p-2-menthen-1-ol
DB-5	1147= 1147	(n=1)	verbenol
DB-5	1155≈ 1151	(n=3)	camphene hydrate
DB-5	1163≈ 1161	(n=5)	pinocarvone
DB-5	1181≈ 1178	(n=25)	4-terpineol
DB-5	1187≈ 1185	(n=8)	p-cymen-8-ol
DB-5	1189≈ 1187	(n=5)	(Z)-3-hexenyl butanoate
DB-5	1195> 1190	(n=27)	α-terpineol
DB-5	1208> 1203	(n=6)	verbenone
DB-5	1209≈ 1208	(n=4)	trans-piperitol
DB-5	1219≈ 1217	(n=10)	trans-carveol
DB-5	1226= 1226	(n=13)	citronellol
DB-5	1224≈ 1228	(n=10)	nerol

Column 1 indicates the column coating, column 2 gives the RI value from the new data source and the sign indicates whether the value is equal to (=), approximately the same (≈), higher or lower than (›, ‹) the mean value. After the mean value the number of values is given (in brackets) on which the mean value is based.

The example in Table 2 shows that most values in the reference are close to the mean value. The values 1119, 1195 and 1208 are acceptable, but are higher than the mean. Value 1124 is unacceptably lower than the mean. It can be seen that the order of elution for all compounds (except for trans-p-2-menthen-1-ol) is as can be expected from the mean values. This might imply that the identification of trans-p-2-menthen-1-ol is not correct.

Table 3. Comparison of new data with existing RI database

GC phase	RI value New mean	Number of references for mean	Compound
DB-Wax	1047? > 1022	(n=18)	α-pinene
□DB-Wax	1089 > 1066	(n=13)	camphene
□DB-Wax	1139 > 1105	(n=18)	β-pinene
□DB-Wax	1161 > 1119	(n=13)	sabinene
□DB-Wax	1178 > 1158	(n=21)	β-myrcene
□DB-Wax	1191? > 1168	(n=12)	α-phellandrene
□DB-Wax	1204? > 1181	(n=17)	α-terpinene
□DB-Wax	1214 ≈ 1197	(n=31)	limonene
□DB-Wax	1259? > 1209	(n=15)	β-phellandrene
□DB-Wax	1265? > 1238	(n=10)	(Z)-β-ocimene
□DB-Wax	1283? > 1246	(n=18)	γ-terpinene
□DB-Wax	1294 > 1269	(n=21)	p-cymene
DB-Wax	1306 > 1286	(n=20)	terpinolene
□DB-Wax	1311 > 1290	(n=25)	caprylaldehyde
□DB-Wax	1372 ≈ 1367	(n=10)	dimethyl trisulfide
□DB-Wax	1395 > 1369	(n=12)	(E)-3-hexen-1-ol

Table 3 shows another example of comparing new data with the database. Except for two values (1214 and 1372), all others are higher than the mean value. Many values (marked with ?) are even unacceptably higher. However, it cannot be concluded that the identification of all these compounds is not correct. Also in this example the order of elution of all these compounds is what can be expected from the mean values. Possibly the coating properties of the column used are different from the average DB Wax coatings or the RI values may have been miscalculated.

Conclusions

- The compilation of the most possible RI values from literature references has proven to be useful and to give more reliable (mean) RI values
- The range of RI values from different literature references for compounds on apolar column coatings is less than on polar coatings
- Especially when RI values from polar coatings are used as references, the reliability of these values has to be examined.

References

1. Nijssen, L.M., Ingen-Visscher, C.A. van, Donders, J.J.H. TNO Triskelion (2014): Volatile Compounds in Food (VCF) database version 15.2, www.vcf-online.nl