

KOVATS INDICES SIMULATION IN ESSENTIAL OIL ANALYSIS

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Kovats indices of essential oils constituents chromatographed on apolar columns of methyl silicones may be estimated by a computer program based on the least squares linear regression that uses the retention times of a few known compounds in the chromatogram and compatibles Kovats indices from literature. Simulated and experimental Kovats indices for an essential oil are compared.

INTRODUCTION

Computer library search is an widespread method used in compound identification in GC/MS analysis of volatiles. It consists in matching a set of reference coded mass spectra against the mass spectrum of the analyte usually called unknown¹⁻¹³. However, the analysis of essential oils based exclusively on mass fragments comparisons needs to be complemented by another criterium of identification because compounds having mono and sesquiterpenes skeletons show very similar mass spectra differing sometimes in minor qualitative aspects¹³.

An possible option to solve this problem is to pre-select from the whole MS library a narrow window of compounds having close Kovats indices¹⁴⁻¹⁵. This way involves some difficulties:

- The existing data on retention time is limited^{16,17} when compared to those available from catalogues of standard mass spectra^{18,19};
- The chromatographic retention time is more sensitive to small experimental variations than the fragmentation of molecules produced by electronic impact at 70 eV.

To minimize these difficulties, it was developed a procedure for simulating Kovats indices using experimental retention times in the chromatogram under analysis and the corresponding compiled Kovats indices from other experiments obtained in very similar conditions.

DISCUSSION AND RESULTS

The methodology used in the simulation involved the following steps:

- An essential oil of known chemical composition was repeatedly chromatographed in different days, in the same experimental conditions, to obtain averaged retention times for to every compound present in the oil. The retention times of a few of these compounds unequivocally identified were associated to their Kovats indices obtained from other experiments or from literature;
- The retention times selected (RT) and the corresponding Kovats indices (KI) were plotted respectively as x and y

coordinates to verify if the functional correlation between the two variables fit into the equation of the straight line (I) below:

$$KI = B \cdot RT + A \quad (I) \text{ where,}$$

B = slope of the straight line with y-axis

A = intercept of the line with the y-axis

RT = retention times selected

KI = Kovats indices corresponding to RT's selected

The solution of equation (I) even with very compatible experimental data rarely originates points lying exactly on the same straight line. Usually the plots obtained do scatter around an ideal line that may be estimated by method of least squares linear regression using equations like II, III and IV^{20,21} below:

$$B = \frac{\sum(KI \cdot RT) - (\sum KI \cdot \sum RT)}{\sum(RT^2) - (\sum RT)^2/N} \quad (II)$$

$$A = \sum KI/N - B \cdot \sum RT/N \quad (III)$$

$$S = \frac{\sum KI^2 - A \cdot \sum KI - B \sum KI \cdot RT}{N - 2}^{0.5} \quad (IV)$$

were,

B = slope of the straight line in equation (I).

A = intercept of the straight line in equation (I).

KI = Kovats indices.

RT = retention times.

N = Number of peaks selected in the analyte.

S = Error distribution in the simulation procedure.

The validity of the results of the simulation is largely dependent on the magnitude of the distribution of the error S due to scattering and should be kept between the limits of 0.0 and 3.0 standard deviations as close as possible to the 0.0 limit.

Table 1 presents the retention times of alpha-pinene, alpha-humulene and guaiol in the chromatogram of Fig. 1 (represented by averaged figures as stated before) and the corresponding Kovats indices. These data were used for calculating the regression line.

$$IK = RT \times 31.84 + 786.32 \quad (S=2.76) \quad (V)$$

Table 2 compares experimental Kovats indices of compounds found in the chromatogram of Fig. 1 with the corresponding simulated values calculated by equation V.

Table 1. Reference compounds and analytical data used to construct the regression line¹ for simulation of Kovats indices of *Croton sonderianus*

RT(2)	KI(2)	COMPOUND(3)
4.31	927	alpha-Pinene
20.59	1443	alpha-Humulene
25.00	1587	Guaiol

(1) Regression line: $IK = RT \times 31.84 + 786.32$, $S = 2.76$

(2) Peaks retention times and compounds from chromatogram of Fig. 1 for calculating the regression line $KT = B \times RT + A$

(3) Experimental Kovats indices from other chromatograms

CONCLUSION

The experimental and simulated values in table 2 are close each other from less than 1%. This means that simulated values are good enough for the purpose of using them as a pre-selection routine in MS library searches, specially in cases when experimental values are not known.

Table 2. Simulated and experimental Kovats indices of compounds identified in the essential oil of *Croton Sonderianus* (F0015¹)

Peak	RT ²	Compound	KI1 ³	KI2 ⁴	Error %
01	4.31	alpha-Pinene	927	927	0.00
02	4.57	Camphene	937	935	0,21
03	5.28	beta-Pinene	965	958	0.73
04	6.71	dl-Limonene	-	1004	-
05	17.27	Ylangene	1341	1339	0.22
06	17.71	alpha-Cubebene	1348	1353	0.30
07	18.29	alpha-Copaene	1369	1372	0.22
08	18.87	Cyperene	1300	1391	0.07
09	19.57	beta-Caryophyllene	1414	1412	0.14
10	19.86	Thujopsene	-	1422	-
11	20.18	C15H24	-	1432	-
12	20.59	alpha-Humulene	1443	1445	0.14
13	20.80	trans-beta-Farnesene	-	1452	0.28
14	21.37	gamma-Murolene	1464	1470	0.41
15	21.47	gamma-Cadinene	1473	1476	0.20
16	22.12	alpha-Murolene	1485	1492	0.47
17	22.22	ar-Curcumene	-	1497	-
18	22.51	C15H24	-	1506	-
19	22.81	delta-Cadinene	1511	1517	0.40
20	25.01	Guaiol	1591	1586	0.31

1. Computer code of the chemical analysis

2. Retention times (min) on the chromatogram of Fig. 1

3. Experimental Kovats indices in the conditions described in the text

4. Kovats indices simulated by equation $IK = RT \times 31.84 + 786.32$

- Experimental values not determined

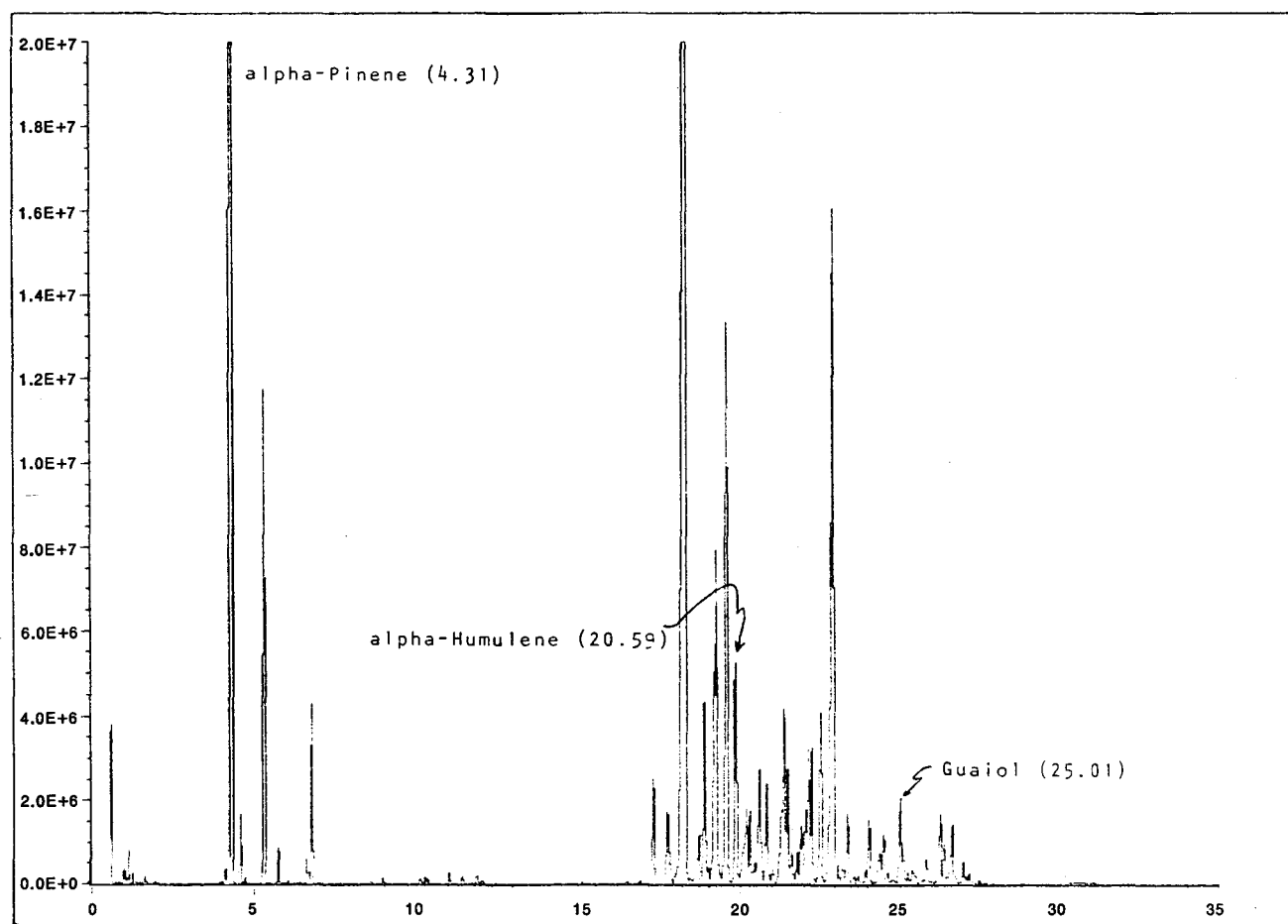


Fig. 1. Chromatogram of Essential Oil of *Croton sonderianus* (F0015). App.: GC/MS Mod. HP 5995, Column: SP2100, 50 m fused silica. Gas flow 1 ml/min, Temp. progr.: 100-300° C at 6° C min. Int. Temp. 250° C.

EXPERIMENTAL

The chromatograms used were obtained on a GC/MS 5971—a Hewlett Packard instrument under the following conditions:

Column: HP.5 crossed 5% phenyl-methyl silicone (25 m, 0.32 mm id. 0.52 μ m of thickness).

Carrier gas: Helium (1 ml/min).

Temperature: Injection port-220°C.

Programming: 50-180° C at 4° C/min then 180-250° C at 10° C/min.

The mass spectra were produced by electronic impact at 70 eV.

The Kovats indices were calculated by co-chromatography using a mixture of n-alkanes as internal standards.

The compound identifications were made by computer library search based on matchings of MS fragments and Kovats indices followed by visual comparison of the mass spectra.

The simulation programs were processed in a IBM-PC compatible microcomputer Prologica (Brazil).

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