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KOVATS' INDICES AS A PRESELECTION ROUTINE IN MASS SPECTRA LIBRARY SEARCHES OF VOLATILES

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The fastest technique for identifying an unknown compound using mass spectrometry involves comparing its mass spectrum with known mass spectra from a collection of standards. Comparisons are usually carried out by a variety of algorithms implemented in computer languages for matching simplified or full mass spectra against library entries (1-14).

One important aspect to be considered is the time consumed by the computer during compound identification. Usually it is impossible to save time in this process in at least three ways: (a) implementation of the programs in machine language; (b) codification of the mass spectra in simplified forms; or (c) selection of the entries in "windows" in the library using preselection routines.

The objective of the present work was to test the importance of the inclusion of gc retention data in mass spectral library search programs. To meet this goal, the same library search program was tested in two alternative ways: (a) compound identification by matching the ten most significant peaks (*m*/*z* vs. intensity) of the unknown against each library entry and (b) the same comparison as above using a preselection routine including a window of Kovats' indices.

The inclusion of Kovats' indices in the analysis of volatiles is easily justified because in gc-ms experiments, retention times and ms fragments are acquired simultaneously and because Kovats' indices are the most widespread form to express relative gc retention times using internal standards (15).

In addition, identifying an unknown by its mass spectrum may be more conclusive when other experimental data are included (16). Therefore, a combination of Kovats' indices with mass spectral search routines could reduce computer processing time and increase the precision of the identification.

The major difficulty in incorporating Kovats' indices into a ms library system lies in the fact that gc retention times are more sensitive to instrumental variations and experimental conditions than ms fragmentation. Therefore, it is possible to have good mass spectra catalogues covering 20,000-30,000 compounds (17,18), but the same is not true for Kovats' indices, even for as few as 2,000 compounds (19). Furthermore, because of the variations in the experimental conditions, published Kovats' indices for the same compound usually show such large discrepancies that compiling large libraries of ms spectra using these retention data is almost impossible. Thus, people using gc/ms techniques usually run several samples per day and obtain hundreds of spectra with the corresponding retention times. If the gc/ms runs are performed using internal standards and standardized conditions, Kovats' indices may be easily calculated. Applying this approach, 60 gc/ms analyses of volatiles were conducted in our laboratory on essential oils and cracked triglycerides using *n*-alkanes as internal standards, and allowed Kovats' indices calculations for a list of 200 compounds. A selection of these results is shown in Table 1, which compares the chemical composition of *Eucalyptus staigeriana* F.v.M., *Piper nigrum* L., and *Citrus limonia* Osbeck analyzed using two different programs: (a) BUSC.SIG, which matches unknown *m*/*z* and relative intensities against library entries

TABLE 1. Essential Oil Analysis Using Two Library Search Programs: BUSC (without Preselection) and BUSKO (with Kovats' Indices Preselection)

Plant	Compound	Identification Time (seconds)	
		BUSC Program	BUSKO Program
E. staigeriana (leaves)	α-Pinene	3.75	0.55
	Sabinene	4.49	0.56
	β-Pinene	4.75	0.58
	Myrcene	5.14	0.65
	Limonene	5.62	0.84
	γ-Terpinene	4.80	0.55
	α-Terpinolene	5.86	0.72
	Methyl Geranate	6.00	0.60
	Neryl Acetate	6.10	0.83
	Geranyl Acetate	6.12	0.85
P. nigrum (leaves)	Eugenol	3.99	0.40
	Methyl-eugenol	3.84	0.42
	α-Humulene	3.34	0.43
	δ-Cadinene	3.95	0.47
C. limonia[a] (leaves)	β-Pinene	4.00	0.30
	Myrcene	3.98	0.29
	Limonene	3.89	0.30
	Citronellal	4.01	0.32
	Neral	4.12	0.31
	Geranial	4.08	0.31
	Neryl Acetate	4.15	0.32
	Geranyl Acetate	4.12	0.32
	Caryophyllene	4.07	0.55

[a] Collected in Pacatuba-Ce-Brazil. File No. 381 in Departamento de Quimica Orgânica e Inorgânica.

and (b) BUSKO.SIG, which matches *m/z* and intensities in the same way as the BUSC.SIG program, but within a window of ±40 units of Kovats' indices.

From the results shown it can be seen that compound identifications achieved by the two programs were the same in the three examples given, but the time consumed for identification using Program BUSKO.SIG was almost ten times shorter than that using Program BUSC.SIG. It is important to emphasize that the experimental library has only 200 entries, and it is easily seen that, for a number of entries substantially greater than 200, the time wasted in matching the unknown against the whole library would be much longer. However, for obvious reasons, the time consumed using the program with the preselection routine will not be substantially affected.

In summary, three samples of essential oils from *Eucalyptus staigeriana, Piper nigrum,* and *Citrus limonia,* were analyzed by gc-ms using library search programs with and without Kovats' indices as a preselection routine. The results showed that identifications were the same in the examples given but computer times consumed using the preselection routine were reduced by a factor of approximately ten.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All gc-ms runs were performed on a HP 5995 Hewlett Packard instrument equipped with a capillary fused silica SP 2100 column (30 m) using helium as carrier gas (1 ml/min) and temperature programmed at 50°-250°, 4°/min.

SAMPLES.—The samples used were essential oils extracted from northeastern Brazilian plants or cracked triglycerides from Brazilian vegetable oils.

COMPUTER PROGRAMS.—Computer programs were implemented in BASIC and developed on terminal TVA 1052 for a DEC 10 system. They were used to calculate Kovats' indices and to perform library search compound identification.

BUSC.SIG PROGRAM.—This program was the same as described before (12) but used mass spectra simplified to the ten most significant peaks (selected by *m/z* and rel. int.).

BUSKO.SIG PROGRAM.—This program used the same mass spectra simplification process as the BUSC.SIG program and a preselection routine based on Kovats' indices instead of the procedure described before (20).

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