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MULTIVARIATE STATISTICAL TREATMENT OF PLANT EXTRACT COMPOSITIONAL DATA: AVERAGE MASS SCAN OF THE TOTAL ION CHROMATOGRAM (AMS) APPROACH[†]

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Abstract. *It was recently confirmed that relative abundances of* m/z *values of the average mass scan of the total GC chromatograms (AMS) are suitable variables for multivariate statistical comparison (MVA) of essential oils. These are even more applicable, reliable and faster than the traditionally used variables–percentages (peak areas) of individual oil constituents. Herein, we have explored if AMS-derived variables are appropriate for MVA comparison of plant solvent extract compositional data. To achieve this, average mass scans of the total GC chromatograms and chemical compositions (relative percentages) of eight diethyl ether extracts (six different species; samples were analyzed using GC-FID and GC-MS; data from the literature) were separately compared using two MVA methods: agglomerative hierarchical clustering analysis and principal component analysis. The obtained results strongly suggest that MVA of complex volatile mixtures (GC-MS analyzable fractions of plant solvent extracts), using the corresponding AMS, could be considered as a promising time saving tool for easy and reliable comparison purposes. The AMS approach gives comparable or even better results than the traditional method.*

Key words: *Principal component analysis, hierarchical clustering analysis, average mass scan of the total ion chromatogram, diethyl ether extract, plant volatiles*

1. INTRODUCTION

Multivariate statistical analysis (MVA) has been repeatedly used to reveal evolutionary relationships among different plant species or track storage effects in the case of economically and/or pharmacologically important plant extracts/essential oils [1-6]. Two MVA analyses, most frequently employed in this sense, are the principal component analysis (PCA) and the agglomerative hierarchical cluster analysis (AHC) [1-7]. The most

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usual (later on termed "traditional" or "classical") approach to MVA analyses of the plant derived mixtures utilizes percentages of single compounds or sometimes summed percentages of structurally and/or biogenetically related compounds from TIC or FID chromatograms as MVA variables, while the samples (botanical mixtures) represent the observations among which (dis)similarity is sought after [1-7].

However, the "classical" MVA-GC-FID/MS approach suffers from several shortcomings [1,7]. Firstly, it could be extremely time consuming. Secondly, problems with identification and peak resolution are quite frequent. We have recently shown that AMS (average mass scans of the total ion chromatograms) profiles−these are readily available from standard GC-MS instruments (Fig. 1)−of essential oils [1], or artificial complex volatile mixtures [7], may be used as a source of new MVA variables. Such variables, *m/z* values of AMSes, appear to be less prone to errors frequently occurring in the identification process, require less time than the "traditional" approach and could even possibly give a novel insight into the relationships among samples being compared [1,7]. Additionally, AMS profiles provide an additional set of variables that may serve as a control [1,7].

In this study, we set our goal to probe the applicability of the AMS approach for MVA comparison of plant solvent extracts aimed to reveal evolutionary/taxonomic relationships. To achieve this, average mass scans of GC chromatograms and chemical compositions of 8 diethyl ether extracts obtained from 6 taxa belonging to different plant genera/families (literature data [8-12]; Table 1) were separately compared using AHC and PCA.

2. MATERIALS AND METHODS

2.1. Plant species and preparation of solvent extracts

The list of analyzed samples is given in Table 1. Details on the identity and origin of plant materials and procedures applied for the preparation of solvent (diethyl ether) extracts are given in the original references [8-12].

2.2. Gas chromatography and gas chromatography mass spectrometry

GC-MS analyses of all samples were repeated three times using a Hewlett-Packard 6890N gas chromatograph. The gas chromatograph was equipped with a fused silica capillary column DB-5MS (5% phenylmethylsiloxane, 30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies, Palo Alto, USA) and coupled with a 5975B mass selective detector from the same company. The injector and interface were operated at 250 ° and 315 °C, respectively. The oven temperature was raised from 70 ° to 315 °C at a heating rate of 5 °C/min and then isothermally held for 20 min. As a carrier gas helium at 1.0 mL/min was used.

Table 1 The list of analyzed samples and selected time intervals ([Rt_1-Rt_2]) from the corresponding GC-MS runs used to generate AMS profiles

Sample	Rt_1 (min)	Rt_2 (min)	Plant species [reference]
E1	2.20	30.76	*Artemisia abrotanum* L. [8]
E2	2.22	51.79	*Ramonda serbica* Panč. [9]
E3	2.20	51.28	*R. nathaliae* Panč. et Petrov. [9]
E4	2.20	32.01	*Foeniculum vulgare* Mill. (root extract) [10]
E5	2.15	44.23	*F. vulgare* (schizocarp extract) [10]
E6	2.16	44.50	*Vitis vinifera x* (*V. labrusca x V. riparia*) [11]
E7	2.26	61.58	*Lonicera fragrantissima* Lindl. & Paxton (extraction period: 1 day) [12]
E8	2.23	61.55	*L. fragrantissima* (extraction period: 20 days) [12]

Table 2 Dominant constituents of the analyzed samples [8-12]

Compound	E1[a]	E2	E3	E4	E5	E6	E7	E8
1,8-Cineole	10.5				0.2			
Terpinolene				6.5				
Fenchone					13.3			
Borneol	6.0							
Ascaridole	13.1							
Geraniol							5.9	7.1
Geranial							1.4	4.6
(*E*)-Anethole				tr	66.1			
Germacrene D	6.5							
Presilphiperfolan-9α-ol	4.8							
Silphiperfol-5-en-3-one A	14.6							
Dillapiole				77.5				
Loganetin							19.1	2.1
Palmitic acid		5.8	3.3	0.1				1.0
α-Bisabolol oxide A acetate	8.7							
Linoleic acid (syn.[b] (*Z*,*Z*)-9,12-octadecadienoic acid)		6.0	7.2					0.6
(*E*,*Z*)-9,12-Octadecadienoic acid			4.8					2.8
Ethyl oleate						12.4		
Pentacosane		0.2	0.3		tr	8.3		tr
Heptacosane		0.6	0.3		0.1	15.0	0.3	0.2
Squalene (all *E*)		36.0	59.4					
Nonacosane	0.3	1.2	0.2		0.2	11.0	15.0	14.4
Hexyl docosanoate							5.6	5.2
10-Nonacosanone					5.8			
Hentriacontane		1.2				15.0	0.9	1.1
10-Nonacosanol							18.1	24.1
β-Sitosterol		22.7	10.8	0.2				
Hexyl tetracosanoate							7.6	5.1
Stigmast-4-en-3-one		4.7	3.3					
Hexyl triacontanoate							5.0	6.8

[a]Designations of the samples were defined in Table 1. [b]syn.-synonym.

The samples, 1 μL of the corresponding diethyl ether extract solution in diethyl ether (1 : 100, w/v), were injected in a pulsed split mode (the flow was 1.5 mL/min for the first 0.5 min and then set to 1.0 mL/min throughout the remainder of the analysis; split ratio 40 : 1). Mass selective detector (MSD) was operated at the ionization energy of 70 eV, in the 35–500 amu range with a scanning speed of 0.34 s. GC (FID) analyses were carried out under the same experimental conditions using the same column as described for the GC-MS. The percentage composition was computed from the GC peak areas without the use of correction factors. Qualitative analyses of the sample constituents were carried out as described in original references [8-12]. The main constituents of these samples are listed in Table 2.

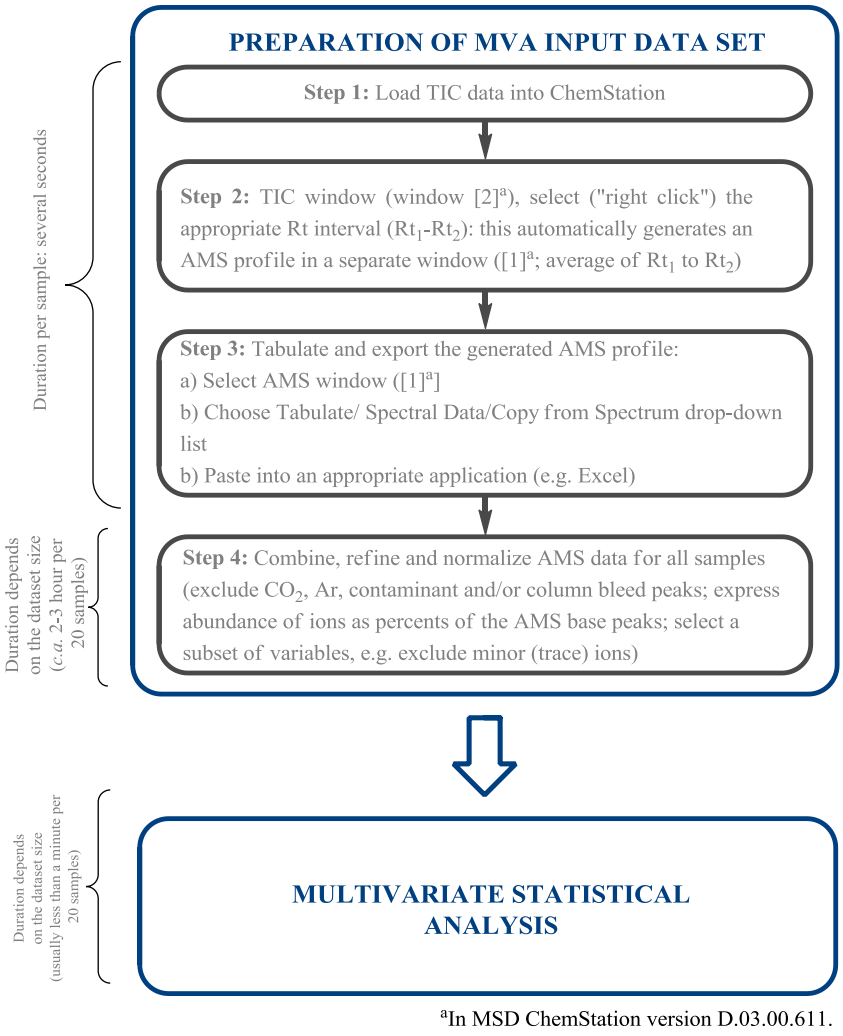

Fig. 1 Flow diagram showing steps needed to acquire an AMS-MVA input dataset

2.3. Average mass scans (AMS) of the total ion chromatograms of the analyzed samples

Average mass scans of total ion chromatograms (AMS) of all analyzed samples (8 in total) were created by ChemStation Software (MSD ChemStation D.03.00.611, Agilent Technologies: Spectrum/Tabulate/Spectral Data) as an average of Rt_1 (2.15-2.26) to Rt_2 (30.76-61.68) min and present a set of arithmetic average values of abundances of ions recorded by a mass selective detector in the given timeframe, rounded to a nominal mass (35-500 amu). Large solvent peaks appearing up to a Rt of 2 min were not recorded. The duration of a single run was 69 min. After Rt_2 (30.76-61.68 min) no ions corresponding to the last peak apex of the given GC chromatogram were detected and the interval between Rt_2 – 69.00 min was not taken into account to lessen the effect of column bleed peaks. The relative abundances of AMS *m/z* peaks, that account for both the relative abundances of ions in individual mass spectra, as well as the relative percentages of the corresponding oil components [1,7], are given in percentages, with the value of 100% assigned to the most abundant peak in every AMS, and the percentages of all other peaks given relative to the AMS base peak. The *m/z* values that corresponded to column bleed peaks (*m/z* 281 and 207), carbon dioxide (*m/z* 44), and argon (*m/z* 40, contaminant from the carrier gas) were excluded. In Table 1, Rt_1 and Rt_2 values for all samples are given. Table 3 lists the *m/z* values and their relative abundances that corresponded to the characteristic peaks from the AMS profiles of the analyzed samples. The flow diagram showing steps needed to acquire AMS profiles of the analyzed samples and the AMS-MVA input dataset is given in Fig. 1.

2.4. Multivariate statistical analysis

Principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) analysis were performed using an Excel program plug-in XLSTAT, version 2010.03.5 (Addinsoft). Unsupervised PCA and AHC (instead of their supervised counterparts) were used herein as these two are applied much more frequently for such purposes. Two different sets of variables were subjected to both methods: percentages of the extracts' components obtained from GC experiments (their mean values; only constituents with percentages higher than 1%, in at least one sample, were taken into account) and AMS variables (average mass scan of the total GC chromatogram relative abundance of *m/z* values; only *m/z* with relative abundances higher than 1%, in at least one sample, were taken into account). AHC was determined using Euclidean distance where the aggregation criterion was Ward's method. Determination of the number of statistically different classes was performed by using an automatic truncation option of XLSTAT Software. The results show the groups to which each observation belongs. The automatic truncation is based on the entropy and tries to create homogeneous groups. When the increase in dissimilarity level is strong, a level has been reached where groups are grouped that are already homogenous. Automatic truncation uses this criterion to decide when to stop aggregating observations (or groups of observations). PCA of the Pearson (n) type was performed.

3. RESULTS AND DISCUSSION

3.1. Theoretical background

Let m be the total number of mass scans (S) recorded during a single GC-MS run (if the duration of the total run (when the MSD is turned on) is t and the time necessary to record one scan Δt, then $m=t/\Delta t$; in this study, t=67 min, Δt=0.34 s and m=11823), and let n be the maximum number of m/z values (mass to charge ratios) observable in a single S ($n \leq 465$ if the MSD is operated in the 35–500 amu range). Also, let y_{ij} be used to designate the response of the MSD for the ion(s) with the mass to charge ratio $(m/z)_j$, recorded during the mass scan S_i. Then, the mass scan S_i (i=1,2,…,m), could be given as a collection (set) of pairs of m/z values and the corresponding responses of the MSD [1]:

$$S_i=\{[y_{i1,}\,(m/z)_1],\,[y_{i2,}\,(m/z)_2],...,\,[y_{in},\,(m/z)_n]\} \tag{1}$$

In the same time, the average mass scan of the total ion chromatogram of a GC-MS run (analysis) could be represented as follows:[1]

$$\text{AMS} = \{[\overline{y}_1,\,(m/z)_1],\,[\overline{y}_2,\,(m/z)_2],...,\,[\overline{y}_n,\,(m/z)_n]\}=\{[(y_{11}+y_{21}+...+y_{m1})/\text{m},\,(m/z)_1],\,[(y_{12}+y_{22}+...+y_{m2})/\text{m},\,(m/z)_2],...,\,[(y_{1n}+y_{2n}+...+y_{mn})/\text{m},\,(m/z)_n]\} \tag{2}$$

Here, $\overline{y}_j$ stands for the average response of the MSD for a single $(m/z)_j$ ion current, over the whole scanning period t (all recorded scans (m in total) were taken into account) [1]:

$$\overline{y}_j = \frac{\sum_{k=1}^{m} y_{kj}}{m} \tag{3}$$

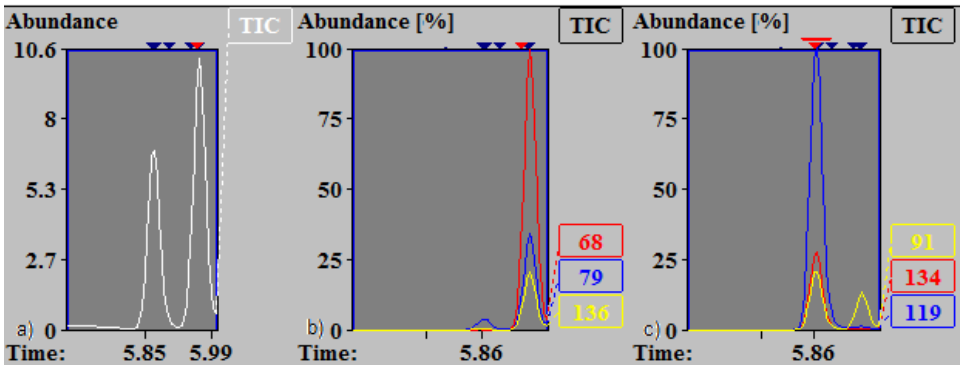

Fig. 2 a) Part of a GC (TIC) chromatogram of *F. vulgare* schizocarp oil [10] (Rt=5.80-6.00 min) with two well resolved peaks, *p*-cymene (peak apex at Rt 5.87 min) and limonene (peak apex at Rt 5.96 min), and changes of selected partial ion currents with time: b) m/z 68, 79 and 136 (the dominant ions in the MS of limonene) [13] and c) m/z 91, 119 and 134 (the dominant ions in the MS of *p*-cymene) [13].

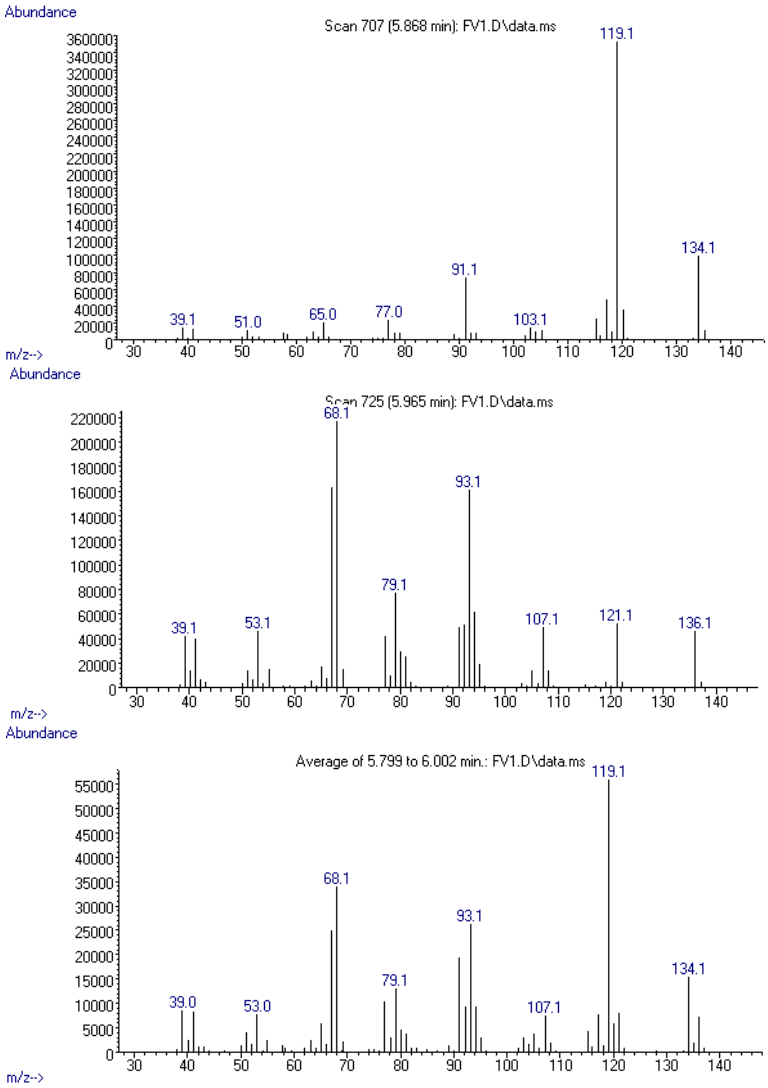

Fig. 3 Mass scans from a GC (TIC) chromatogram of *F. vulgare* schizocarp oil [10]: a) Rt 5.87 min and b) Rt 5.96 min; c) and corresponding AMS profile generated over the Rt (min) interval [5.80, 6.00]

The AMS represents a non-normalized superposition of individual MSes: it includes data on both the qualitative and quantitative composition of the analyzed sample (mixture) [1,7].

The following example could be used to illustrate this. Fig. 2 shows a part of a GC-MS chromatogram (Rt=5.80-6.00 min) of the essential oil obtained from schizocarps of *F. vulgare* [10]. Apart from the TIC, this figure also shows changes of selected partial ion currents (*m/z* 68, 79, 91, 119, 134 and 136) with time. Two observable peaks correspond

to *p*-cymene (peak apex at Rt 5.87 min) and limonene (peak apex at Rt 5.96 min). Mass scans recorded at Rt 5.87 and 5.96 min, as well as the AMS generated over the Rt interval [5.80, 6.00] are given in Fig. 3. Figs 2b, 2c, 3a and 3b clearly show that the contribution of different partial ion currents to TIC is not equal (different abundances). Partial ion current of the fragments at *m/z* 119 are dominant during the elution of *p*-cymene, for example [13].

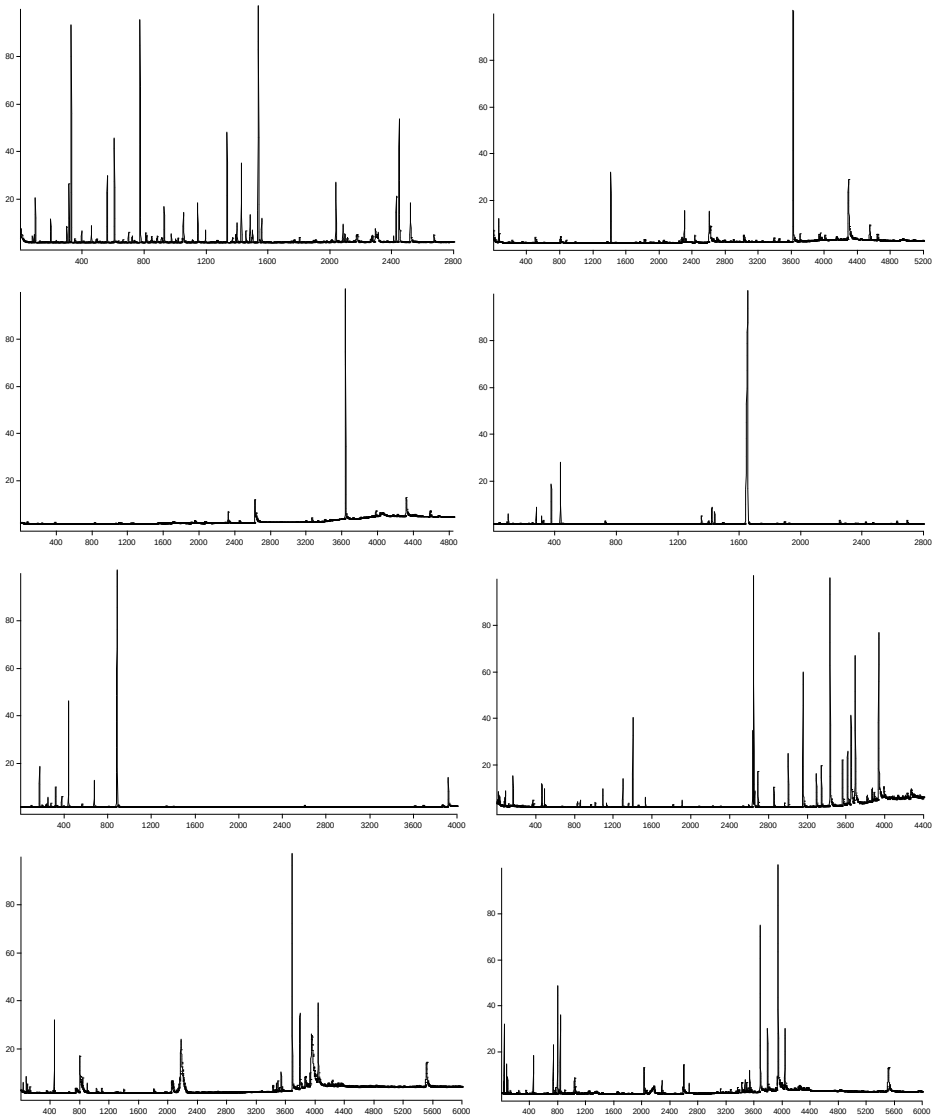

Fig. 4 TIC chromatograms of the analyzed extracts [8-12] (E1-E8; top to bottom, left to right)

Similarly, the most abundant ion during the elution of limonene is that at *m/z* 68 [13]. MSD (mass selective detector) responses (TIC abundances) at Rt 5.87 and 5.96 min were 1 400 000 and 900 000, respectively. These represent the sum of the partial ion currents for all detected ions (*e.g.* approximately 360 000, 100 000 and 220 000 for ions at *m/z* 119, 134 (Rt 5.87 min) and 68 (Rt 5.96 min), respectively). In the case of the mentioned limonene and *p*-cymene ions, the averaged MSD responses, observable from the AMS formed over the Rt interval [5.80, 6.00] (Fig. 3c), are significantly lower than the same from the MSes recorded at Rt 5.87 or 5.96 min (e.g. approximately 55 000, 15 000 and 35 000 for ions at *m/z* 119, 134 and 68). This is in agreement with equations (2) and (3): the overall (summed) MSD response for a single *m/z* over the AMS interval is divided by the total number of scans during the chosen Rt interval.

Table 3 The dominant *m/z* values (rel. int., %) in the AMS profiles of the analyzed samples

m/z	E1	E2	E3	E4	E5	E6	E7	E8
41	40	80	86	6	13	47	85	82
43	100	75	71	5	6	84	100	95
55	26	74	90	5	9	55	71	71
57	3	55	52	2	4	100	99	83
67	15	44	69	4	7	16	21	35
69	22	100	100	4	18	33	92	100
71	19	26	27	1	4	62	69	59
73	0	32	42	0	2	11	65	33
77	23	11	23	22	31	2	9	11
79	27	25	43	13	22	5	10	19
81	26	67	86	4	35	13	24	32
83	11	29	37	5	2	22	44	53
85	5	10	16	1	2	41	36	38
91	53	34	34	22	24	6	10	16
93	41	20	29	24	17	1	16	16
95	41	35	53	2	2	7	13	23
96	6	19	33	1	2	15	90	34
97	11	20	28	0	1	19	48	43
117	5	8	16	5	30	1	3	8
119	35	13	16	7	3	1	1	8
121	61	16	20	21	21	1	5	6
133	11	16	21	6	24	2	50	16
147	3	10	17	4	55	0	1	7
148	4	1	2	1	100	0	1	2
177	5	3	22	33	0	0	1	5
191	1	9	13	6	1	2	57	15
209	0	7	11	0	1	2	65	18
222	11	0	0	100	0	1	0	0
253	0	7	10	0	1	1	44	14
282	0	2	9	0	1	0	39	14

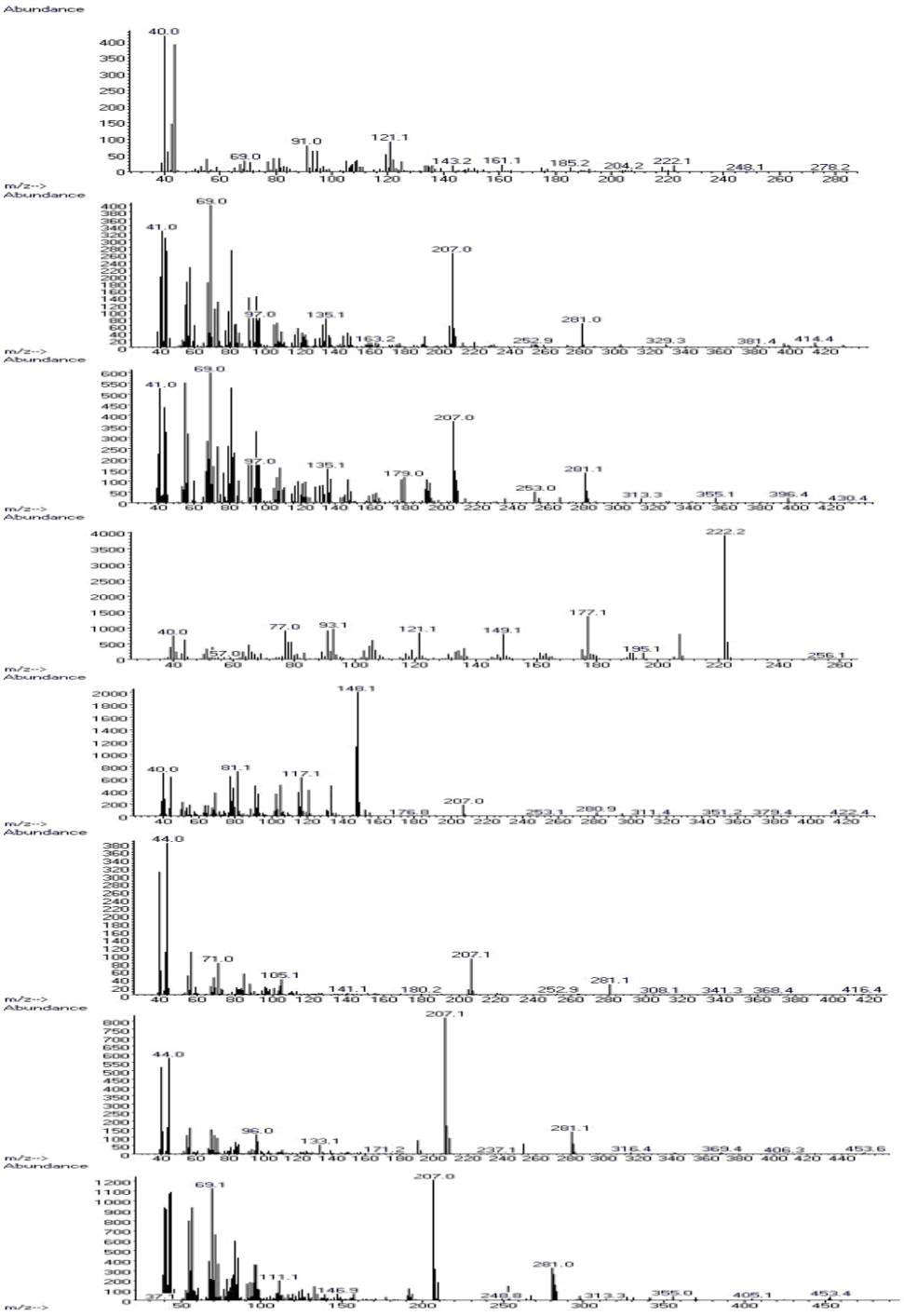

Fig. 5 AMS profiles of the analyzed extracts (E1-E8; top to bottom)

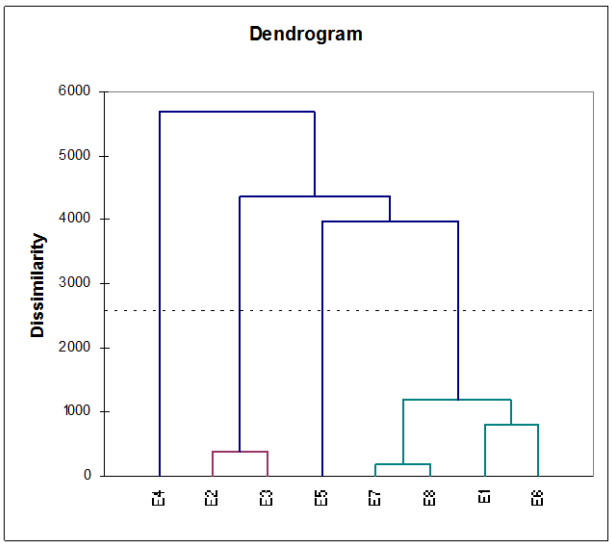

Fig. 6 Dendrogram (AHC1 analysis) representing chemical composition dissimilarity relationships of E1-E8 samples (observations) obtained by Euclidian distances dissimilarity within the interval [0, 5703], using aggregation criterion-Ward's method. Four groups of samples were found (from left to right): C1-C4.

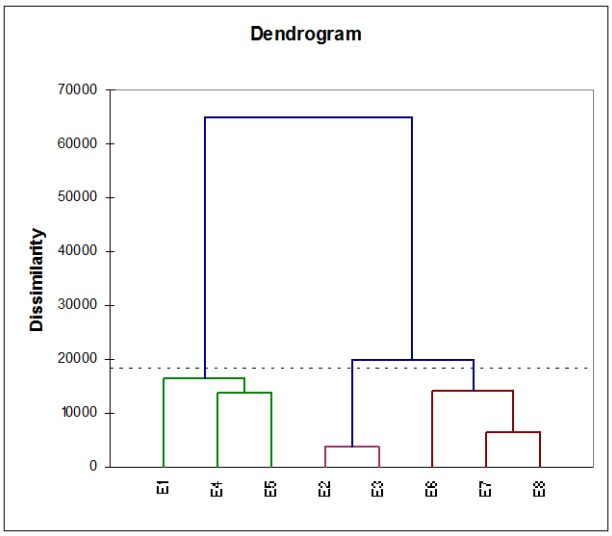

Fig. 7 Dendrogram (AHC2 analysis) representing total GC chromatogram average mass scans (AMS *m/z* values as variables) dissimilarity relationships of E1-E8 (observations) obtained by Euclidian distance dissimilarity within the interval [0, 65105], using aggregation criterion-Ward's method. Three groups of samples were found (from left to right): C5-C7.

3.2. AMS profiles in MVA analyses of plant solvent extracts

Applicability of the relative abundances of *m/z* values of AMSes in a MVA treatment of plant solvent extracts' compositional data (GC analyzable fraction) was tested on a group of 8 samples [8-12] (E1-E8; Table 1). The selection of these samples was not a random one - it contains several subgroups characterized with high level of "internal homogeneity" that are, however, mutually quite different (e.g. E2 and E3; E7 and E8). Around 200 different compounds were detected and identified in the 8 samples [8-12]. Only 73 of them (those with the relative amount ≥1% in at least one sample) were used for MVA analyses. To make the discussion easier, the main constituents of the mentioned samples are given in Table 2. The corresponding TIC chromatograms are depicted in Fig. 3. AMS profiles of these samples (both tabular and graphical representations) are given in Table 3 and Fig. 5.

The results of the AHC analyses (AHC1: original variables (percentage composition) and AHC2: AMS variables (relative abundances of *m/z* values)) are given in Figs. 6 and 7. A certain level of similarity of the extracts' clustering, based on both performed AHC analyses, should be noted, Figs. 6 and 7. For example, both dendrograms placed samples E2 and E3 (*R. nathaliae* and *R. serbica* extracts) [9] within a single separate class (C2, Fig. 6 and C6, Fig. 7). Chemical compositions of these samples were mutually very similar in respect to the amount of their most dominant compounds (e.g. squalene, β-sitosterol, palmitic and stearic acids, Table 2).

As expected [1,7], the dominant *m/z* values in the AMSes of samples E2 and E3 corresponded to *m/z* values of the most abundant ions in the mass spectra of the major contributors of the mentioned extracts [13]. Similar was true for samples E7 and E8 (two *L. fragrantissima* extracts) [12]. Nevertheless, two *F. vulgare* samples [10] (E4 and E5)–these were obtained from the roots and schizocarps of the very same plant population–were, according to the results of AHC1, statistically separated (classes C1 and C3). E4 and E5 had different major constituents (Table 2): (*E*)-anethole and dillapiole, but both of these were closely related in a biosynthetic sense (phenylpropanoids). Unfortunately, the "traditional" MVA approach disregards information on the same biosynthetic origin of the constituents (variables) [1]. A possible way how to go around this problem is to use relative amounts of biosynthetic classes of components as variables; however, this approach is also time consuming [6]. The clustering based on the AMS variables (AHC2) recognized relatedness of E4 and E5 (subclade of C5). It seems that even the subtle (at least at the first glance) similarities in the fragmentation patterns of their main constituents (e.g. similar ratios of the rel. intensities of *m/z* 77 and 91 ions from MSes of (*E*)-anethole and dillapiole) [13], further transposed to E4/E5-AMS profiles, were sufficient for the segregation of these two samples.

Botanically and chemically (Table 2) speaking, the aromatic (essential-oil rich) species *A. abrotanum* (E1) [8] is not related to *L. fragrantissima* (E7, E8) [12], *Vitis* sp. hybrid (E6) [11] or *F. vulgare* (E4, E5) [10]. Thus, the placement of E1 (AHC1, AHC2) is rather strange. Nevertheless, AHC2 (AMS approach) placed E1 together with the samples obtained from other aromatic species (*F. vulgare*).

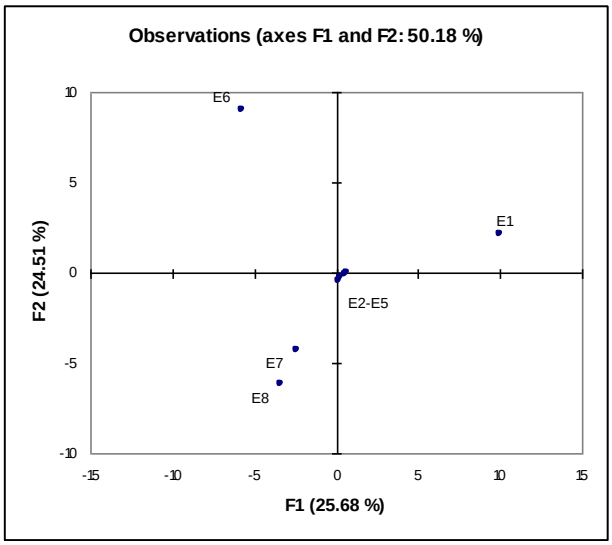

Fig. 8 Principal component analysis (PCA1: original variables (percentage composition)) of E1-E8. Axes (F1 and F2 factors-the first and second principal component) refer to the ordination scores obtained for the samples. Axis F1 accounts for *ca.* 26% and axis F2 accounts for a further 24% of the total variance.

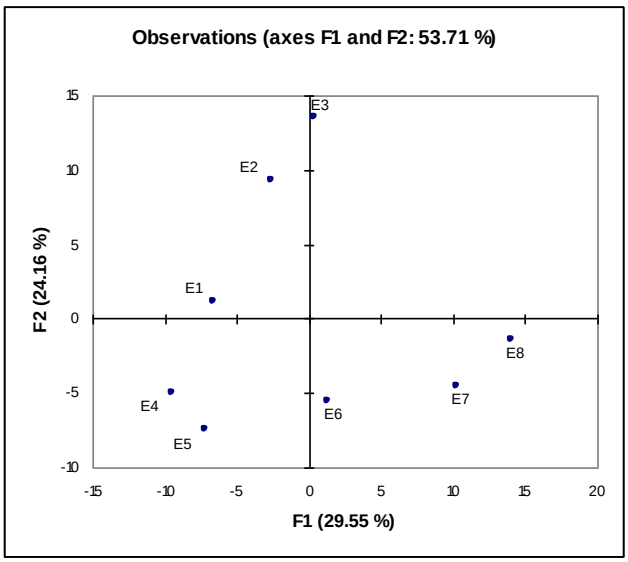

Fig. 9 Principal component analysis of E1-E8 (PCA2: AMS variables). Axes (F1 and F2 factors-the first and second principal component) refer to the ordination scores obtained for the samples. Axis F1 accounts for *ca.* 30% and axis F2 accounts for a further 24% of the total variance.

Surprisingly, according to AHC1 (the traditional approach), terpene-rich E1 is most similar to E6 (diethyl ether washings of intact *V. vinifera* x (*V. labrusca* x *V. riparia* grapes), dominated by alkanes [1,11].

The results of PCA analyses (PCA1: traditional approach; PCA2: AMS approach) are given in Figs 8 and 9. The results of PCA were in fair agreement with those obtained from the corresponding AHC analyses.

4. CONCLUSION

Herein, we have shown that there is a fair level of accordance between the results of MVA comparison of diethyl ether extracts (GC analyzable fraction) compositional data performed using the traditional and AMS-type variables. The observed discrepancy between the obtained results should not be considered as either erroneous or illogical. In fact, to a certain level, the results of the two MVA analyses (different sets of variables) reflect different features but those that are in common between the compared oils. It was already observed that in the case of MVA comparison of essential-oil compositional data[1] when an incoherent group of samples (comprised of several subgroups characterized with a high level of internal homogeneity, but that are mutually unrelated) was subjected to MVA, the AMS approach gave much better, evolutionarily more logical results. Herein presented results are in complete agreement with this.

To conclude, the average mass scans of the total GC chromatograms of mixtures of plant volatiles enable their fast, reliable and easy MVA comparisons. Usage of *m/z* values of AMSes enabled significant shortening of the time needed for pre-MVA operations (from several days to several hours, Fig. 1) and avoided detailed time-consuming analyses of complex mixtures: plant extracts or essential oils. This could be particularly important when a great number of samples have to be screened and compared.

REFERENCES

1. N.S. Radulović, P.D. Blagojević, Average mass scan of the total ion chromatograms: a new GC-MS derived variable for fast and reliable multivariate statistical treatment of essential oil compositional data, *Journal of Chromatography A*, **1301**, 190-199 (2013).
2. P.A.R. Ramanoelina, J. Viano, J.P. Bianchini, E.M. Gaydou, Occurrence of various chemotypes in Niaouli (*Melaleuca quinquenervia*) essential oils from Madagascar using multivariate statistical analysis, *Journal of Agricultural and Food Chemistry*, **42** (5), 1177-1182 (1994).
3. R. Karousou, E. Hanlidou, D. Lazari, Essential oils of *Micromeria dalmatica* Benth., a Balkan endemic species of section Pseudomelissa, *Chemistry and Biodiversity*, **9** (12), 2775-2783 (2012).
4. A. Bouzabata, V. Castola, A. Bighelli, L. Abed, J. Casanova, F. Tomi, Chemical variability of Algerian *Myrtus communis* L. *Chemistry and Biodiversity*, **10** (1), 129-137 (2013).
5. D.V. Lakušić, M.S. Ristić, V.N. Slavkovska, J.B. Šinžar-Sekulić, B.S. Lakušić, Environment-related variations of the composition of the essential oils of rosemary (*Rosmarinus officinalis* L.) in the Balkan Peninsula, *Chemistry and Biodiversity*, **9** (7), 1286-1302 (2012).
6. N.S. Radulović, B.K. Zlatković, R.M. Palić, G.S. Stojanović, Chemotaxonomic significance of the Balkan *Achillea* volatiles, *Natural Product Communications*, **2** (4), 453-474 (2007).
7. N.S. Radulović, P.D. Blagojević, D. Skropeta, Average mass scan of the total ion chromatogram vs. percentage chemical composition in multivariate statistical comparison of complex volatile mixtures, *Journal of the Brazilian Chemical Society*, **21** (12), 2319-2326 (2010).

8. N.S. Radulović, P.D. Blagojević, B.K. Zlatković, R.M. Palić, Volatile profile of *Artemisia abrotanum* L. (Asteraceae) from Serbia, *South African Journal of Chemistry*, **62**, 30-32 (2009).
9. N.S. Radulović, P.D. Blagojević, R.M. Palić, B.K. Zlatković, B.M. Stevanović, Volatiles from vegetative organs of paleoendemic resurrection plants *Ramonda serbica* Panč. and *Ramonda nathaliae* Panč. et Petrov., *Journal of Serbian Chemical Society*, **74** (1), 35-44 (2009).
10. N.S. Radulović, P.D. Blagojević, A note on the volatile secondary metabolites of *Foeniculum vulgare* Mill. (Apiaceae), *Facta Universitatis: series Physics, Chemistry and Technology*, **8** (1), 25-37 (2010).
11. N.S. Radulović, P.D. Blagojević, R.M. Palić, Volatiles of the grape hybrid variety Othello (*Vitis vinifera* x (*Vitis labrusca* x *Vitis riparia*)) cultivated in Serbia; *Journal of Essential Oil Research*, **22** (6), 616-619 (2010).
12. N.S. Radulović, P.D. Blagojević, R.M. Palić, Composition of diethyl ether flower extracts of *Lonicera fragrantissima* Lindl. & Paxton (Caprifoliaceae), *Natural Product Communications*, **4** (11), 1581-1585 (2009).
13. R. Adams, *Identification of essential oil components by gas chromatography/mass spectrometry*, Allured Publishing Corporation, Carol Stream, 4th ed., 2007.

MULTIVARIJANTNO STATISTIČKO POREĐENJE HEMIJSKOG SASTAVA BILJNIH EKSTRAKATA: USREDNJENI MASENI SKEN UKUPNOG JONSKOG HROMATOGRAMA, UMS-PRISTUP

Nedavno je pokazano da se relativne zastupljenosti m/z *vrednosti usrednjenih masenih skenova ukupnih jonskih hromatograma (UMS) mogu koristiti kao varijable pri multivarijantnoj statističkoj analizi (MVA) etarskih ulja. Ovaj tip varijabli se lakše primenjuje, daje pouzdanije i brže rezultate nego tradicionalne promenljive - procenti (površine ispod pikova) pojedinačnih sastojaka analiziranih ulja. U ovom radu smo ispitivali primenjivost UMS-varijabli za MVA poređenje hemijskog sastava odabranih biljnih ekstrakata. Da bi ovo postigli, usrednjeni maseni skenovi ukupnih jonskih hromatograma i hemijski sastavi (procentualna zastupljenost) 8 dietil-etarskih ekstrakata (6 različitih biljnih vrsta; ekstrakti su analizirani korišćenjem GC-FID i GC-MS analiza; literaturni podaci) su zasebno upoređivani korišćenjem dve MVA metode: hijerarhijske klaster analize i analize glavne komponente. Dobijeni rezultati ukazuju na to da se MVA analiza složenih smeša isparljivih (pod uslovima GC-MS analize) jedinjenja, zasnovana na primeni UMS varijabli, može koristiti za njihovo lako, brzo i pouzdano poređenje. UMS pristup daje uporedive, ili čak bolje rezultate u odnosu na tradicionalni metod.*

Ključne reči: *Analiza glavne komponente, hijerarhijska klaster analiza, usrednjeni maseni sken ukupnog jonskog hromatograma, dietil-etarski ekstrakt, isparljiva biljna jedinjenja*