Acta Poloniae Pharmaceutica – Drug Research, Vol. 72 No. 3 pp. 517–525, 2015 ISSN 0001-6837
Polish Pharmaceutical Society

APPLICATION OF CHEMOMETRICS FOR IDENTIFICATION OF PSYCHOACTIVE PLANTS

ANNA PAULINA KOWALCZUK[1]*, ANNA ŁOZAK[1], MONIKA KILJAN[1], KRYSTYNA MĘTRAK[1] and JORDAN KORDIAN ZJAWIONY[2]

[1]National Medicines Institute, Chełmska 30/34, 00-725 Warszawa, Poland
[2]Department of Pharmacognosy and Research Institute of Pharmaceutical Sciences, School of Pharmacy, The University of Mississippi, University MS 38677, USA

Abstract: Drug market changes dynamically causing many analytical challenges for police experts. Among illicit substances there are synthetic designer products but also herbal material. Plant material is usually in fine-cut or powdered form, thus difficult to identify. For such fragmented material classic taxonomical identification methods using anatomical and morphological features of the plant cannot be employed. The aim of the study was to develop an identification method of the powdered material with employment of multidimensional data analysis techniques. Principal Component Analysis (PCA) was chosen as a method of data exploration. The study was conducted on four plants controlled in Poland: *Salvia divinorum*, *Mitragyna speciosa*, *Psychotria viridis* and *Calea zacatechichi*. The compatibility of grouping features of selected species was compared in two variants: chemical and elemental composition. In a first variant, GC-MS chromatograms of extracts were analyzed and in the second, elements composition with the AAS and the ICP-MS techniques. The GC-MS method, based on the qualitative interpretation of results, allows for clear differentiation of samples with regard to their species affiliation. Even the plants belonging to the same family *Rubiaceae*, *P. viridis* and *M. speciosa* formed homogeneous and clearly separated clusters. Additionally, the cluster analysis was performed, as a method confirming sample grouping.

Keywords: narcotic plants, chemometrics, identification, gas chromatography, elements

Narcotics available on illegal market often contain psychoactive plant material. In many cases this material derives from species illegal in particular country. Plant material is usually in fine-cut or powdered form, thus difficult to identify. For such fragmented material classic taxonomical identification methods using anatomical and morphological features of the plant cannot be employed (1). For plants, in which psychoactive compound is species-specific, identification includes botanical and phytochemical analysis. Identity of *Salvia divinorum* Epling et Játiva-M. (*Lamiaceae*) and *Mitragyna speciosa* Korthals (*Rubiaceae*) can be confirmed by detection of secondary metabolites, salvinorin A and mitragynine, respectively (2). In a case where the psychoactive compound is not specific to the particular species, such as N,N-dimethyltryptamine for *Psychotria viridis* Ruíz et Pavón (*Rubiaceae*) (3), unambiguous determination whether a test material is derived from an illegal plant, may be problematic. The biggest problem, however, like in case of *Calea zacatechichi* Schlechtendal (*Asteraceae*), is the identification of the plant, in which no psychoactive substance or the other marker was found (4).

The aim of the study is to develop a method enabling for identification of plant species controlled according to Polish law, based on their chemical characteristics without the need for determination of specific compounds. Chemometric techniques have such potential. They allow building a classification model, in which objects are grouped according to their taxonomic assignment of the species. The data on which the model is constructed derived from the characteristics of the plant. The features used in this technique may consist of micromorphological (5, 6), chemical (7-9) or elemental composition of plant (10). Identification of species using chemometric techniques is becoming more and more popular, but so far it has not been used for the assessment of illegal plant products.

* Corresponding author: e-mail: apkowalczuk@gmail.com, tel. 22 841 06 23 (383)

Table 1. Mineralization parameters of sample preparation for ASA and ICP-MS analyses.

Step	Time of heating (s)	Time of cooling (s)	Microwave power (W)
1	120	120	87.5
2	120	600	157.5
3	120	120	210.0
4	120	600	245.0
5	120	120	280.0
6	300	600	315.0

Table 2. Conditions of the F-AAS method.

Element	Flame type	Analyte wavelength [nm]	Internal standard	Working range [mg/L]
Ca	Nitrous oxide/acetylene	422.7	yes	0.25 – 1.25
Fe	Air/acetylene	248.3	no	0.5 – 6
K	Air/acetylene	766.5	no	0.1 – 1.0
Mg	Air/acetylene	285.2	yes	0.1 – 0.5
Na	Air/acetylene	589.0	no	0.25 – 1.0
Zn	Air/acetylene	213.9	no	0.1 – 1.0

Table 3. Eigenvalues of the principal components obtained from GC-MS results.

	Eigenvalues	% of the total variance	Accumulated eigenvalues	Accum. - % of the total variance
1	52.26088	18.02099	52.2609	18.0210
2	43.31437	14.93599	95.5752	32.9570
3	32.18627	11.09871	127.7615	44.0557
4	22.84326	7.87699	150.6048	51.9327
5	16.63155	5.73502	167.2363	57.6677
6	15.22790	5.25100	182.4642	62.9187
7	14.41694	4.97136	196.8812	67.8901
8	13.27539	4.57772	210.1566	72.4678
9	12.03532	4.15011	222.1919	76.6179
10	11.25389	3.88065	233.4458	80.4985
11	9.38060	3.23469	242.8264	83.7332
12	8.02784	2.76822	250.8542	86.5015
13	7.24924	2.49974	258.1034	89.0012
14	7.11811	2.45452	265.2216	91.4557
15	5.60138	1.93151	270.8229	93.3872
16	4.69090	1.61755	275.5138	95.0048
17	3.97270	1.36990	279.4865	96.3747
18	3.68404	1.27036	283.1706	97.6450
19	3.36614	1.16074	286.5367	98.8058
20	2.18997	0.75516	288.7267	99.5609
21	1.27333	0.43908	290.0000	100.0000

The specific aims of this study is to use the chemometric techniques to identify narcotic plants in their powdered form. The study was conducted on four plants controlled in Poland: *S. divinorum*, *M. speciosa*, *P. viridis* and *C. zacatechichi*. We compared the compatibility of grouping features of selected species in two variants: chemical and elemental composition. In a first variant, GC-MS chromatograms of extracts were analyzed and in the second, elements composition using AAS (Ca, Fe, K, Mg, Na, Zn) and the ICP-MS techniques (Ag, Al, B, Ba, Be, Cd, Ce, Co, Cr, Cs, Cu, Dy, Eu, Ga, Gd, Hg, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sm, Sr, U, V). Choosing the right test, with data characterizing the material according to the taxonomic assignment of the species, distinct clusters can be obtained. The optimal grouping allows for the construction of the classification model as a new tool for plant material identification in forensic laboratories.

EXPERIMENTAL

Plant materials

The examined products were purchased from the Internet. According to the provider's declaration the investigated material was: dried shredded leaves of *Salvia divinorum* from Mexico - four samples S; dried leaves of *Psychotria viridis* from Hawaii - sample P(H), two samples from Brazil – P(B) and three samples from Peru – P(P); dried shredded or powdered leaves of *Mitragyna speciosa* from Thailand – three samples M(T) and three samples from Bali – M(B); and dried shredded herb of *Calea zacatechichi* from Mexico – five samples C.

Reagents and materials

Methanol, chloroform, ethyl acetate, dichloromethane and hexane pure p.a. were purchased from POCH (Poland). Deionized water was obtained with

Table 4. PC1. PC2 and PC3 values obtained from GC-MS results corresponding to the plant samples: S – *S. divinorum*; P(H) – *P. viridis* from Hawaii; P(B) – *P. viridis* from Brazil; P(P) – *P. viridis* from Peru; M(T) – *M. speciosa* from Thailand; M(B) – *M. speciosa* from Bali and C – *C. zacatechichi*.

Sample	PC1	PC2	PC3
S	0.4738	1.9206	0.3141
S	0.4596	1.8023	0.3572
S	0.5656	1.9082	0.2824
S	0.4685	2.2157	0.5758
P(H)	0.4896	-0.5476	-1.2652
P(B)	0.4669	-0.4627	-1.1980
P(B)	0.4532	-0.4247	-1.1696
P(P)	0.4964	-0.0875	-1.4251
P(P)	0.5246	-0.1882	-1.1493
P(P)	0.4716	-0.3891	-1.2706
P(P)	0.4170	-0.3511	-1.5186
M(T)	0.6054	-0.8545	1.2147
M(T)	0.5585	-0.6953	1.0609
M(T)	0.6610	-0.8655	1.1713
M(B)	0.5683	-0.8936	1.1969
M(B)	0.6053	-0.8676	1.1051
M(B)	0.6461	-0.9753	1.3668
C	-2.1012	-0.0014	0.3661
C	-1.8771	-0.1466	0.1522
C	-1.9034	0.0014	0.0370
C	-1.5961	-0.1167	-0.0250
C	-1.4535	0.0192	-0.1790

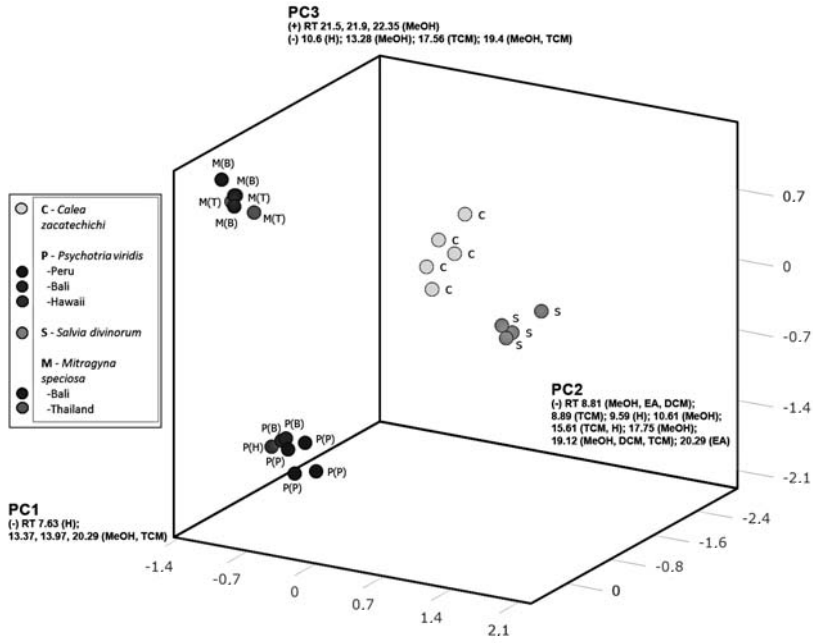

Figure 1. PC1, PC2 and PC3 scores for GC-MS results according to the species: S – *S. divinorum*; P(H) – *P. viridis* from Hawaii; P(B) – *P. viridis* from Brazil; P(P) – *P. viridis* from Peru; M(T) – *M. speciosa* from Thailand; M(B) – *M. speciosa* from Bali and C – *C. zacatechichi*

Table 5. Eigenvalues of the principal components obtained from elemental composition.

	Eigenvalues	% of the total variance	Accumulated eigenvalues	Accum. - % of the total variance
1	10.67761	30.50745	10.67761	30.5075
2	5.66157	16.17591	16.33918	46.6834
3	3.88256	11.09304	20.22174	57.7764
4	3.75177	10.71934	23.97351	68.4957
5	2.50624	7.16069	26.47975	75.6564
6	2.35411	6.72603	28.83386	82.3825
7	1.24418	3.55479	30.07804	85.9373
8	1.15646	3.30418	31.23450	89.2414
9	0.89630	2.56085	32.13080	91.8023
10	0.82497	2.35705	32.95577	94.1593
11	0.50425	1.44071	33.46002	95.6000
12	0.40901	1.16861	33.86903	96.7686
13	0.34960	0.99886	34.21863	97.7675
14	0.26917	0.76906	34.48780	98.5366
15	0.20557	0.58735	34.69337	99.1239
16	0.10717	0.30621	34.80054	99.4301
17	0.08818	0.25195	34.88873	99.6821
18	0.05438	0.15537	34.94311	99.8375
19	0.02698	0.07707	34.97008	99.9145
20	0.01904	0.05440	34.98913	99.9689
21	0.01087	0.03107	35.00000	100.0000

deionizating filter system Water PRO PS from Labanco (Kansas City, USA). Membrane filters Chromafil Pet-45/25 from Macherey-Nagel GmbH & Co.KG (Germany).

ICP-MS and AAS reagents: stock solution IV-ICPMS-71A: Ag, Al, As, B, Ba, Be, Cd, Ce, Co, Cr, Cs, Cu, Dy, Eu, Ga, Gd, Mn, Nd, Ni, Pb, Pr, Rb, Se, Sm, Sr, Tl, U, V in 10.00 µg/mL from Inorganic Ventures (USA); standard solution of: Ca, Fe, Hg, In, K, Li, Mg, Mo, Na, Sb, Zn in 1000 µg/mL from Merck (Germany); nitric acid of ICP grade from Merck (Germany); lanthanum nitrate of AAS grade from Merck (Germany); redistilled water with Nanopure Diamond UV system from Barnstead. Argon 99,999%, nitrous oxide and acetylene min. 99,6% from Air Products.

Apparatus

GC-MS Agilent GC7890A+5975C VLMSD instrument from Agilent Technologies (USA); ICP-MS spectrometer Thermo Electron X Series II from Thermo Electron Corporation (USA); AAS Solaar M6 from Thermo Elemental; hollow cathode lamps: Ca-HCL, Fe-HCL, K-HCL, Mg-HCL, Na-HCL and Zn-HCL; microwave decomposition unit UniClever from Plazmatronika-Service (Poland); vibratory grinder with mill made of corundum from Testchem (Poland) were used

Sample preparation

For GC-MS analysis, powdered dried plant material (0.5 g) was extracted with 5.0 mL of methanol (MeOH), ethyl acetate (EA), dichloromethane (DCM), chloroform (TCM) and hexane (H) in an ultrasound bath for 30 min and filtered through membrane filters.

For ICP-MS and AAS methods, dried plant material was ground in vibratory grinder and the lost of drying in 105°C was determined. One gram sample weights were placed in Teflon crucibles and 5 mL of nitric acid was added. The digestion using microwave energy in a closed system was performed in a six-step system. Mineralized solutions were transferred to volumetric flasks and filled up to 100.0 mL with water. Mineralization parameters are presented in Table 1.

Methods

GC-MS

GC-MS was performed with a 5% phenylmethylpolysiloxane capillary column HP-5MS (30 m × 0.25 mm i.d.; film thickness, 0.25 µm; Agilent J&W Scientific (USA). The temperature program consisted

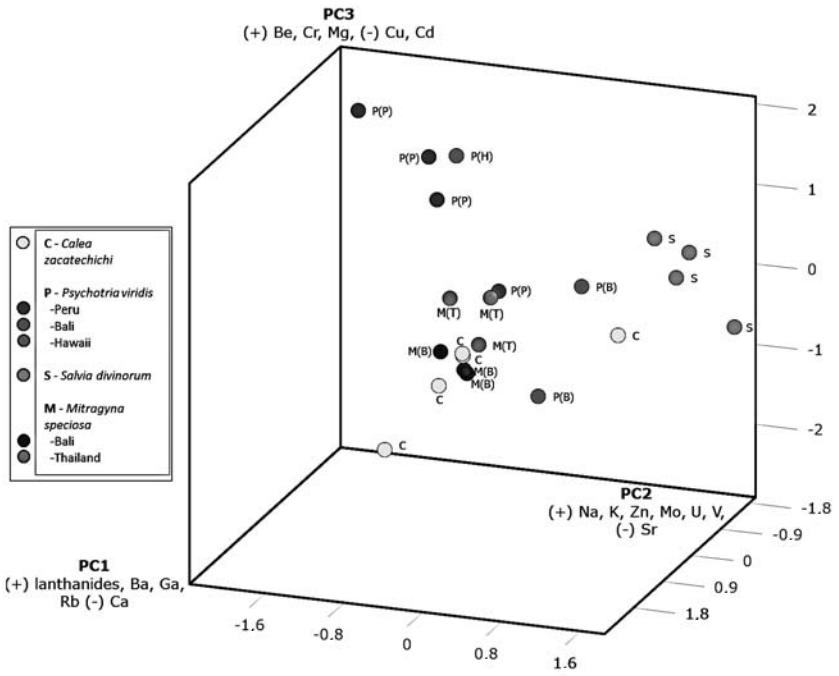

Figure 2. PC1, PC2 and PC3 scores for elemental composition according to the species: S – *S. divinorum*; P(H) – *P. viridis* from Hawaii; P(B) – *P. viridis* from Brazil; P(P) – *P. viridis* from Peru; M(T) – *M. speciosa* from Thailand; M(B) – *M. speciosa* from Bali and C – *C. zacatechich*

of the initial temperature of 100°C held for 0.5 min, followed by a linear ramp up to 300°C at 12°C/min. The inlet temperature was set at 230°C, and the injection was set in the split 1:80 mode. The carrier gas was high-purity helium at a flow rate of 1.0 mL/min.

The MS detector parameters used were: interface temperature, 290°C; ion-source temperature, 230°C; ionization mode, electron ionization (EI); ionization voltage, 70 eV; scan time, 1.0 s/scan; scan range, *m*/*z* 40–550.

ICP-MS

The ICP-MS measurement were performed using the following parameters: R.f. power 1302 W, background line < 0.5 counts/s; gas flow: plasma: 12.4–13,3 L/min; support: 0.83–0.89 L/min, nebulizer: 1.08–1.18 L/min, proportion of double charged ions $^{137}Ba^{2+}/^{137}Ba$ less than 3.0%, proportion of oxide ions $^{156}CeO/^{140}Ce$ less than 3.0%; $^{115}In/^{220}Bkg$ > 800 000; sample aspiration time 30 s; acquisition time for 3 replicates 15 s. The indium solution in 10 µg/L concentration was used as an internal standard.

Content of Ag, Al, B, Ba, Be, Cd, Ce, Co, Cr, Cs, Cu, Dy, Eu, Ga, Gd, Hg, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sm, Sr, U and V has been determined. The measuring range for the elements was 0.5 – 50.0 µg/L.

F-AAS

Content of Ca, Fe, K, Mg, Na and Zn has been measured. The F-AAS method conditions are placed in Table 2.

One milliliter of 5% lanthanum solution was used as an internal standard. The assay of elements was counted on the dry mass of the plant material.

Statistical analysis

For the statistical analysis "Statistica 10" software from StatSoft (USA) was used, all the other calculations were performed in Microsoft Excel.

Table 6. PC1. PC2 and PC3 values obtained from elemental composition corresponding to the plant samples: S – *S. divinorum*; P(H) – *P. viridis* from Hawaii; P(B) – *P. viridis* from Brazil; P(P) – *P. viridis* from Peru; M(T) – *M. speciosa* from Thailand; M(B) – *M. speciosa* from Bali and C – *C. zacatechichi*.

Sample	PC1	PC2	PC3
S	-1.1468	1.0936	0.5144
S	-1.1086	1.4631	0.4082
S	-1.3274	1.8459	-0.5409
S	-1.2050	1.2998	0.0344
P(H)	-0.9381	-0.8459	1.3401
P(B)	1.8894	1.3483	1.0585
P(B)	2.6989	1.1741	-0.0346
P(P)	-0.3715	-0.9399	1.5147
P(P)	0.1871	-0.6727	1.2216
P(P)	-0.1158	-1.5759	2.0946
P(P)	0.2251	-0.0351	0.1863
M(T)	0.5232	-0.0250	0.2139
M(T)	0.2744	-0.2257	-0.4863
M(T)	0.2249	-0.5309	0.0276
M(B)	0.7183	-0.1982	-0.6780
M(B)	-0.1056	-0.7336	-0.7860
M(B)	1.1548	-0.0871	-0.4535
C	-0.3838	0.9778	-0.4295
C	-0.4244	-0.6130	-0.9323
C	0.2709	-1.1794	-1.9426
C	-0.3348	-0.8274	-1.3057
C	-0.7050	-0.7127	-1.0249

The Principal Component Analysis was carried out in two variants, on GS-MS fingerprints obtained from the plant extracts and on the elemental composition of the plants. GC-MS results formed a data matrix composed on 290 variables and 22 cases. In case of the elements assay, the data matrix was composed on 35 variables and 22 cases.

Using PCA algorithm for calculations, the consistent grouping of objects, in both cases, has been achieved and no rotation has been applied.

RESULTS AND DISCUSSION

Species identification of illegal fragmented plant material is a challenging task. Taxonomic research based only on part of the plant is impossible to be performed. Therefore, it seems that the only effective method of identification may be the use of micromorphological examination combined with phytochemical analysis (11). If the substance responsible for psychoactive activity of the plant is known, especially if it is a specific compound, the species identity of the material can be established. Two plants, *S. divinorum* and *M. speciosa* contain active markers, salvinorin A and mitragynine, respectively. In case of *P. viridis*, the psychoactive compound, DMT, is not specific to the species and does not allow the material identification. In *C. zacatechichi*, no psychoactive substance has been recognized so far, and no specific compounds have been isolated.

The aim of the study was to develop an identification method of the powdered material with employment of multidimensional data analysis techniques. Principal component analysis (PCA) was chosen as a method of data exploration. It is a procedure that uses orthogonal transformation of observable variables into a new set of uncorrelated variables (called components). PCA does not involve variable reduction, and the total variance of variables is equal to the sum of variances of principal components (12). Although it would be worthwhile to use the cluster analysis (CA) for variable grouping, our final aim is that the suggested solution from the principal component space be complemented with subsequent cases in the future. The cluster analysis was performed additionally, as a method confirming sample grouping.

A GC-MS studies of the following extracts were performed: methanol, ethyl acetate, dichloromethane, chloroform and hexane, obtained from *S. divinorum*, *P. viridis*, *M. speciosa* and *C. zacatechichi*. Ten retention times corresponding to the largest peak areas were selected from each chromatogram. Each peak represented a single compound corresponding to mass. Retention times of all the peaks were introduced as variables. The sample was characterized on the basis of the presence or absence of a given compound in the extract. As a result, a matrix having 290 columns and 22 rows containing discrete data was obtained. In the examined set, numerous statistically significant correlations at the significance level of $p = 0.001$ were observed. In order to interpret the results, the principal component analysis was performed. Ten principal components (1-10, Table 3) describing 80% of the total number of variances were used for further calculations. The number of principal components for interpretation was selected on the basis of the Cattell's criterion (13), with the assumption that 20% of the given data variance may be omitted with no difference to significant information contained in the data. Principal component: PC1, PC2 and PC3 values obtained from GC-MS results corresponding to each of the plant sample are presented in Table 4.

On the basis of the analysis of certain factor scores value, it was found out that already three first components allow effective differentiation between samples of various species. The first principal component PC1 allows isolation of *C. zacatechichi* samples, the second component PC2 species *S. divinorum* and the third component PC3 differentiates between the species *M. speciosa* and *P. viridis* (Fig. 1).

The PCA of the element composition was performed on the basis of the content of the following metals in the samples: Ag, Al, B, Ba, Be, Cd, Ce, Co, Cr, Cs, Cu, Dy, Eu, Ga, Gd, Hg, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sm, Sr, U and V. In the examined set, numerous statistically significant correlations at the significance level of $p = 0.001$ were observed. In order to interpret the results, the principal component analysis preceded by data standardization was performed. Eight principal components (1-8, Table 5) describing 90% of the total number of variances and meeting the Kaiser criterion were used for further calculations (14). Principal component: PC1, PC2 and PC3 values obtained from elemental composition corresponding to each of the plant sample are presented in Table 6.

The first principal component PC1 differentiates between *S. divinorum* samples and *P. viridis* coming from Brazil (Fig. 2). The species *S. divinorum* and *P. viridis* from Hawaii revealed small amount of lanthanides and a relatively high calcium level, whereas a high level of lanthanides in *P. viridis* samples from Brazil clearly grouped these objects in a separate cluster. Thus, within one *P.*

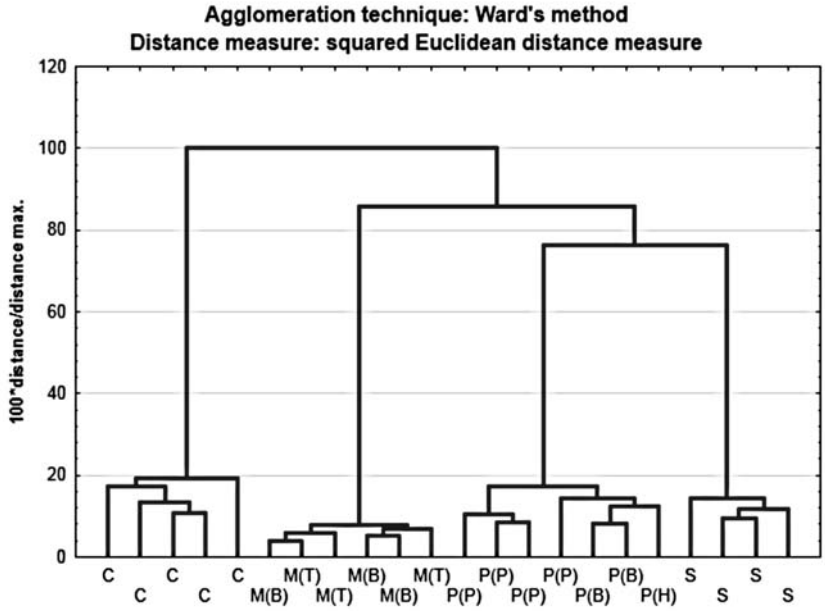

Figure 3. Dendrograms for hierarchical clustering of chemical variables according to the GC-MS chromatograms

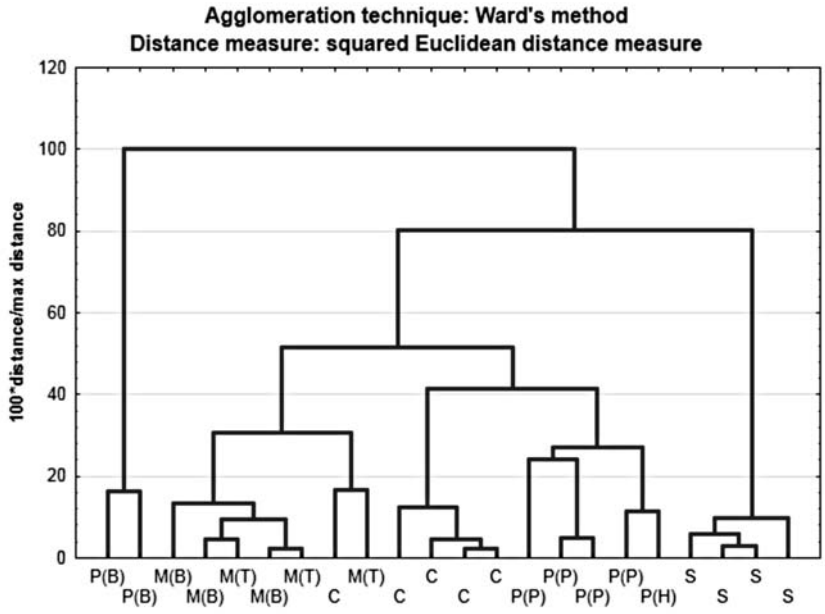

Figure 4. Dendrograms for hierarchical clustering of elemental composition

viridis species, two completely different levels of these metals were observed. The second principal component PC2 differentiates a group comprising *S. divinorum* and *P. viridis* from Brazil *versus P viridis* from Hawaii and Peru and *C. zacatechichi*. *S. divinorum* and *P. viridis* samples from Brazil, in contrast to the second group, contain a larger amount of Na, K, Zn, Mo, U and V, and small amounts of Sr (Fig. 2). The third principal component, PC3 allows isolation of the *P. viridis* group on the basis of a higher

content of Be, Mg, Cr and a low content of Cu and Cd. This component differentiates between *P. viridis* and *C. zacatechichi* (Fig. 2).

The aim of the work was to assess the possibility of using the ICP-MS and GC-MS methods combined with a chemometric analysis for identification of plant narcotics. Sample grouping agreeing with the species affiliation was observed for the results of the GC-MS analysis. Due to low availability of the material, caused by its illegal status, it was impossible to accomplish a model, but the obtained results confirmed the usefulness of PCA for the narcotic plant recognition.

PCA of the results obtained from the ICP-MS and AAS analysis did not lead to formation of clusters representing a single species. This results from the fact that the content of elements in a plant depends on numerous factors. They involve the type of soil and environmental conditions, such as precipitation. In case of endemic plants such as *S. divinorum*, the elemental composition of all the samples was the same. The biggest differences in elements content were observed in *P. viridis*, since the samples came from different places: Hawaii, Brazil and Peru.

The GC-MS method, based on the qualitative interpretation of results, allows for clear differentiation of samples with regard to their species affiliation. Even the plants belonging to the same family - *P. viridis* and *M. speciosa*, formed homogeneous and clearly separated clusters. HCA results presented in dendrograms confirmed that the study of chemical composition is more effective for grouping than the study of elemental composition (Figs. 3 and 4).

CONCLUSIONS

Object grouping, and ultimately a classification model based on the analysis of principal components may be used as a tool for identification of plant narcotics. The method of statistical interpretation of results obtained in the GC-MS analysis of plant-derived extracts presented in this study is an alternative for complex identification procedures involving microscopic methods and phytochemical analysis. Creating a model based on the elemental composition seems to be pointless, due to dependence of the element content of the plant on environmental conditions. However, such a model may be useful for determining the place of origin of the plant material.

The results of this study are an important start in creating a model allowing identification of selected narcotic plants, but they have to be supported with more samples. A good solution may be to initiate cooperation between forensic laboratories in order to provide data and then to use methods based on the chemometric analysis.

Acknowledgment

This work was supported by National Science Center in Kraków, Poland under the grant No. 0240/B/P01/2011/40.

REFERENCES

1. Zuba D., Byrska B., Maciow M.: Anal. Bioanal. Chem. 400, 119 (2011).
2. Babu K.M., McCurdy C.R., Boyer E.W.: Clin. Toxicol. (Phila) 46, 146 (2008).
3. Blackledge R.D., Charlotte M.T.: Microgram J. 18 (2003).
4. Ch. Rätch.: The Encyclopedia of Psychoactive Plants. Ethnopharmacology and Its Application. Park Street Press, Rochester, Vermont 2005.
5. Lakušić B., Lakušić D., Jančić R., Stevanović B.: Flora 201, 108 (2006).
6. Øvstedal D.O., Mjaavatten O.: Plant Syst. Evol. 18, 21 (1992).
7. Zhao Y., Sun J., Yu L.L., Chen P.: Anal. Bioanal. Chem. 405, 4477 (2013).
8. Dale L.M., Thewis A., Boudry C.H., Rotar I., Pacurar F.S., Abbas O., Dardenne P. et al.: Talanta 116, 149 (2013).
9. Sun J., Chen P.: Anal. Bioanal. Chem. 399, 1877 (2011).
10. Lahner B., Gong J., Mahmoudian M., Smith E.L., Abid K.B., Rogers E.E., Guerinot M.L. et al.: Nat. Biotechnol. 21, 1215 (2003).
11. Kowalczuk A.P., Łozak A., Zjawiony J.K.: Forensic Sci. Int. 233, 238 (2013).
12. Stanisz A.: in Accessible course of statistics using STATISTICA PL on examples from medicine. Volume 3. Multivariate analysis. Statsoft Polska, Kraków 2007.
13. Astel A., Obolewski K., Skorbiłowicz E., Skorbiłowicz M.: Desalin. Water Treat. doi: 10.1080/19443994.2014.887491, (2014).
14. Astel A.M., Chepanova L., Simeonov V.: Water Air Soil Pollut. 216, 375 (2011).

Received: 15. 05. 2014