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GROUPING OF RESIDUAL SOLVENTS PRESENT IN PHARMACEUTICALS USING EXPERIMENTAL PLANNING AND CHEMOMETRIC METHODS

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Abstract: The main effects of six experimental factors on the efficiency of HS (headspace) extraction in headspace gas chromatography – flame ionization detector (HS-GC-FID) determination of twenty organic solvents routinely used in production of pharmaceuticals were obtained on the basis of the results of experiments carried out according to the Plackett-Burman factorial design. The effects were used as a basis for grouping the solvents into five groups, the solvents belonging to a group responded similarly to changes of HS conditions. To this end, visualization approaches were used as well as chemometric methods: cluster analysis (CA) and principal component analysis (PCA). Moreover, the most important HS experimental factors were selected for further optimization of the HS-GC determination procedure.

Keywords: headspace, residual solvents, Plackett-Burman factorial design, cluster analysis, principal component analysis

Production of pharmaceuticals is a very demanding and strictly monitored task. Pharmaceutical companies undergo a variety of laws and regulations in order to obtain the best quality products. They have to meet acceptance criteria determined in pharmacopeias or ICH guidelines (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) and executed by regulatory agencies such as FDA (Food and Drug Administration) or EMEA (European Medicines Agency). Among different parameters like identity, assay, content uniformity, dissolution etc. also the control of residual solvents' content is necessary. Residual solvents (RS), according to ICH Q3C guideline (1), are defined as "organic volatile chemicals that are used or produced in the manufacture of drug substances or excipients, or in the preparation of the drug products" which "are not completely removed by practical manufacturing practices". They are divided into three, or precisely four[1], classes on the basis of their toxicological properties and impact on the environment. If the final product or starting materials are suspected of being contaminated with such substances, their content should be evaluated and justified (2).

Every analytical procedure used for this task should be validated. Pharmacopeias propose their own methods (3, 4) based on HS-GC technique. However, when they are used, a single injection lasts about 60 min (without headspace equilibration time), and detection of some solvents is impossible[2]. These disadvantages come from the fact that the methods described in pharmacopoeias are general ones, meaning that they try to compromise analytical and instrumental conditions for all considered solvents. Moreover, for quantitative determinations of residual solvents, methods taken from pharmacopoeias need validation. For this reason, manufacturers try to find their own tests which would be (if possible) quicker, easier and more appropriate for their specific samples and analytes. Many studies have been done in this area. Most of the proposed methods are based on gas chromatography systems (5). Actually, this technique is a natural choice for the residual solvents which are relatively volatile and thermally stable substances. It appears that sample preparation and its introduction to the GC sys-

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[1] For Class IV solvents there is no adequate toxicological data.

[2] Formamide, 2-ethoxyethanol, 2-methoxyethanol, methylene glycol, N-methylpyrrolidone and sulfolane are not detected.

tem are the most demanding stages of the analysis. The most suitable and then frequently used are extraction techniques like static or dynamic headspace extraction (HS), solid phase microextraction (SPME) and single drop microextraction (SDME) (5). Each method offers indirect way of introducing analytes into the chromatograph. Direct injection demonstrates drawbacks like possible contamination of the GC system with possible non-volatile matrix components or complicated sample preparation procedures (6). Decision of using one of the mentioned extraction techniques depends on the sample type, analytes, maximum allowable amount, laboratory equipment and time purposed for the analysis. SPME has a reputation of an alternative to HS (7, 8), but as of now, static HS is a preferred technique for the analysis of residual solvents in bulk pharmaceuticals (9-14) and in the final products (4, 15-19).

The static HS extraction technique is based on partitioning of volatile compounds in a heated closed vial between the sample dissolution medium and the gas phase, followed by the transfer of an aliquot of the vial headspace gas containing the volatile analytes to the GC injector (19). HS principles are relatively simple, however, several experimental factors need to be optimized. Depending on which volatiles components are tested and in what type of samples, parameters such as: kind of dissolution medium, equilibration time and temperature, headspace vial volume, volume of sample solution, presence of matrix, inorganic salt addition and shaking may influence sensitivity, precision, accuracy and detection limits of the method.

Optimization of experimental conditions of determination of a number of analytes simultaneously is a challenge for the analyst as optimum conditions for individual analytes usually differ. It is why finding groups of solvents of similar properties (responding similarly to the changes in experimental factors) is important from the practical point of view. In the presented investigation the grouping was performed using design of experiments as well as chemometric methods including cluster analysis (CA) and principal component analysis (PCA).

EXPERIMENTAL

Reagents and materials

Twenty solvents which are most frequently used in the pharmaceutical industry were taken into account in the presented examinations. Acetone, isopropanol, n-hexane, methyl isobutyl ketone, ethyl acetate, chloroform, methylene chloride and toluene were of gas chromatography grade (Merck, Damstadt, Germany). Methanol, n-heptane, 1,4-dioxane, tetrahydrofuran, ethanol and n-butanol were of spectroscopy grade (Merck). Acetonitrile was of liquid chromatography grade (Merck). Isobutanol, cyclohexane and methyl isobutyl ketone (POCH, Gliwice, Poland), methyl ethyl ketone AR (Park Scientific Ltd., Northampton, U.K.) and benzene (OBR PR, Płock, Poland) were of analytically pure grade and n-propanol was of synthesis grade (Merck). Sodium chloride of analytically pure grade (POCH) was used as a salting out agent. As a matrix, a mix of microcrystalline cellulose, lactose and corn starch in 1:1:1 weight ratio was used.

Chromatographic system and method

The GC system consisted of a model 6890N gas chromatograph equipped with flame ionization detector and headspace sampler G1888 from Agilent Technologies (Palo Alto, CA, USA). The following experimental conditions were used: Detector: FID. Column: DB-624 column (30 m × 0.53 mm × 3 μm) from J&W Scientific Agilent Technologies. Column temperature: 40°C hold 10 min, then raised up to 70°C at the rate of 6°C/min and kept for 4 min, then the temperature raised again to 100°C at the rate of 5°C/min and subsequently to 230°C at the rate of 20°C/min.

Injector temperature: 180°C, detector temperature: 250°C, inlet pressure: 3.68 psi, split ratio: 5 : 1. Starting HS conditions used for linearity tests and for reference measurements (the term reference measurement will be explained in Data analysis section) were as follows: equilibration temperature: 85°C, equilibration time: 30 min, injection volume: 1 mL, shaking: low, dissolution medium: water, sample volume: 5 mL, inorganic salt addition: none, matrix addition: none, vial volume: 20 mL.

Linearity of the method

Linearity of HS-GC-FID responses (Y_i; i = 1, 2,…, 20; see below) *vs.* concentrations of residual solvents were checked using standard solutions in which concentrations of individual analytes assumed the following levels: 40, 50, 100, 200 and 500%, where 100% corresponded to maximum allowable limits according to ICH guideline (1) and pharmacopoeias (3, 4) in assumption that 250 mg of sample was being analyzed (mass transferred to headspace vial). For all solvents, except benzene, determination coefficients (squared correlation coefficients) exceeded 0.99 (0.98 for benzene). The exemplary chromatograms obtained for standard

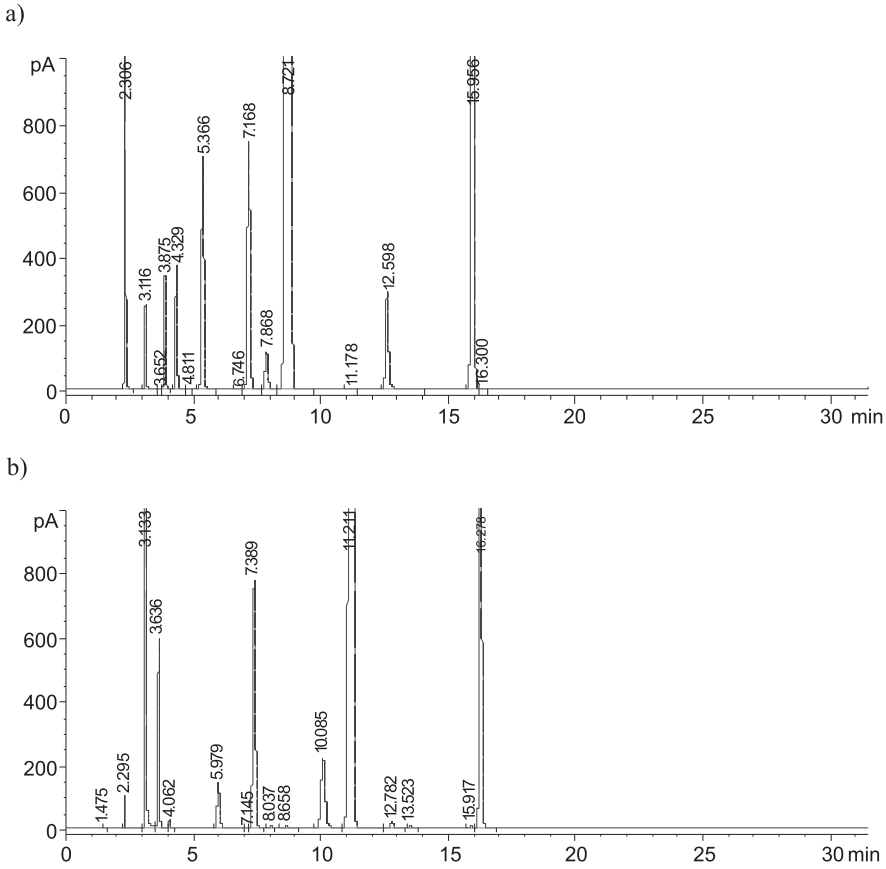

Figure 1. Chromatograms obtained for standard solutions at 100% concentration level. (a) 2.30 min – methanol, 3.12 min – ethanol, 3.88 min – isopropanol , 4.33 min – n-hexane, 5.37 min – methyl ethyl ketone, 7.17 min – methylene chloride, 7.87 min – tetrahydrofuran, 8.72 min – cyclohexane, 12.60 min – n-butanol, 15.95 min – methyl isobutyl ketone and (b) 2.29 min – methanol, 3.13 min – ethanol, 3.64 min – acetone, 4.06 – acetonitrile, 5.98 min – n-propanol, 7.39 min – ethyl acetate, 8.04 – chloroform, 10.09 min – isobutanol, 11.21 min – n-heptane, 16.28 min – toluene

solutions at 100% concentration level are presented in Fig. 1. Two mixtures of solvents were analyzed separately, each mixture containing 10 analytes.

Plackett-Burman factorial design

To recognize which of the headspace parameters have significant influence on the efficiency of extraction process, experiments according to Plackett-Burman (P-B) factorial design were performed. This two-level design is recommended as a very efficient screening tool, when only the main effects are searched.

Response functions and HS factors

The following characteristics (response functions, criterions) of HS extraction efficiency were used, for each solvent individually:

$$Y_i = \frac{A_{i\ sample}}{A_{i\ std}} \quad (1)$$

where $A_{i\ sample}$ – area of chromatographic peak corresponding to analyte "i" measured in current conditions; $A_{i\ std}$ – area of chromatographic peak corresponding to analyte "i" measured in reference conditions.

Reference measurements were carried out in order to eliminate influence of signal changes between experiments performed in different days. To this aim, concentrations of solvents in reference solutions were at their maximum allowed limits (according to ICH guideline Q3R) in assumption that 250 mg of sample was analyzed (mass transferred to headspace vial) and HS conditions were the same as presented above as starting conditions (see above Experimental, Chromatographic system and methods).

The following experimental factors describing HS conditions were taken into account in the present investigations:

X_1 – volume of the liquid phase in HS vial,
X_2 – time of sample conditioning at temperature X_3,
X_3 – temperature of sample conditioning,
X_4 – intensity of sample shaking
X_5 – presence of matrix in a sample
X_6 – salting agent (NaCl) presence in a sample

Determination of factors' effects

The influence of the above factors, X_1, X_2, X_3, X_4, X_5 and X_6, on response Y_i (i indicates analyte) was determined on the basis of the results of measurements of response Y_i carried out in experimental conditions according to the P-B design presented in Table 1, where "-1" and "1" denote, respectively, lower and upper level of a factor. In the present investigation the P-B factorial was completed with the central point of the plan (point 9).

The coded variables, $\tilde{X}_u$ (u = 1, 2,… 6) presented in Table 1 are related to the original factors according to the following formula:

$$\tilde{X}_u = \frac{X_u - X_u^{(0)}}{\Delta X_u} \quad (2)$$

where $X_u^{(0)}$ is the value of factor X_u at the center of the plan and ΔX_u equals half of the difference between upper and lower levels of the factor in the plan (Table 2).

In Table 2 the assumed lower and upper levels of factors are presented in original variables, as well as the coordinates of the central point of the plan in which measurements were carried out additionally to what is normally required in the P-B design.

On the basis of measurements carried out at the experimental points 1-8 of the P-B design the coefficients b_{iu} (u = 1, 2,…6) in the following statistical models were determined:

$$\hat{Y}_i = b_{i0} + b_{i1}\tilde{X}_1 + b_{i2}\tilde{X}_2 + b_{i3}\tilde{X}_3 + b_{i4}\tilde{X}_4 + b_{i5}\tilde{X}_5 + + b_{i6}\tilde{X}_6 + b_{i7}\tilde{X}_7 \quad (3)$$

The effects b_{iu} were determined for all 20 analytes individually. A coefficient b_{iu} is a measure of the main effect of factor u (u = 1, 2,…6) on the response Y_i corresponding to solvent i (i = 1, 2, …, 20).

Table 1. Plackett-Burman experimental design expressed in "coded" variables.

Experimental points (conditions)	Factors							Y_i
	$\tilde{X}_1$	$\tilde{X}_2$	$\tilde{X}_3$	$\tilde{X}_4$	$\tilde{X}_5$	$\tilde{X}_6$	$\tilde{X}_7$	
1	1	1	1	–1	1	–1	–1	Y_{i1}
2	–1	1	1	1	–1	1	–1	Y_{i2}
3	–1	–1	1	1	1	–1	1	Y_{i3}
4	1	–1	–1	1	1	1	–1	Y_{i4}
5	–1	1	–1	–1	1	1	1	Y_{i5}
6	1	–1	1	–1	–1	1	1	Y_{i6}
7	1	1	–1	1	–1	–1	1	Y_{i7}
8	–1	–1	–1	–1	–1	–1	–1	Y_{i8}
9	0	0	0	0	0	0	0	Y_{i0}

Table 2. The assumed levels of factors.

Factor	Factor levels		
	lower (-1)	upper (1)	center (0)
X_1 [mL]	1	5	3
X_2 [min]	10	30	20
X_3 [°C]	45	85	65
X_4	no	high	low
X_5 [mg]	0	300	150
X_6 [%]	0	20	10

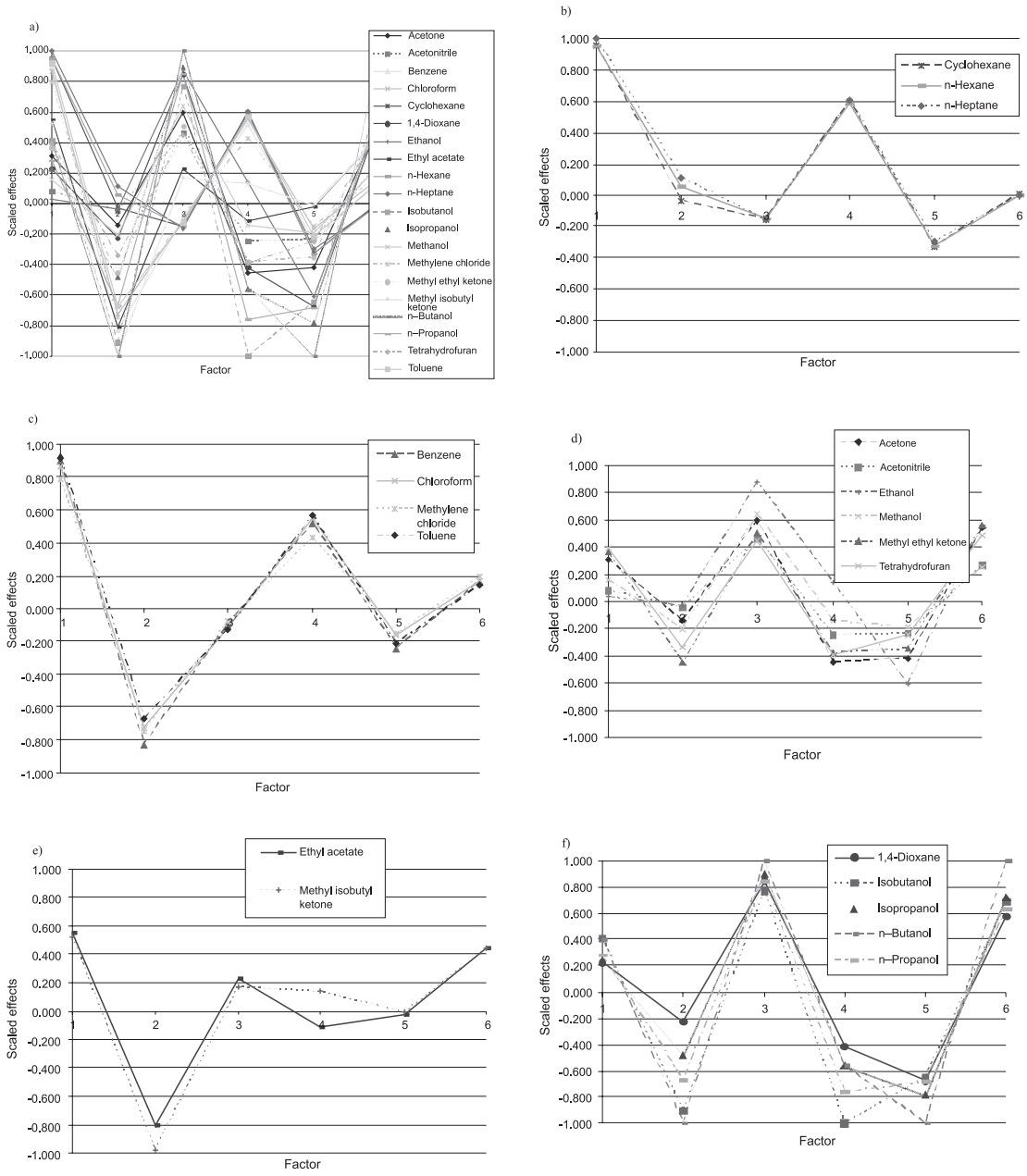

Figure 2. Scaled factor's effects including all solvents (a) and five groups of similar solvents (b-f)

Since for a given analyte i, the effects b were calculated independently from each other (which is a result of orthogonality of the P-B plan) their significance was checked using Student's t-test: $t = |b|/s_b$, where $s_b = s_Y/\sqrt{8}$ and s_Y denotes standard error of the response determination. The error s_Y was estimated (individually for each analyte) as a square root of averaged variance calculated from six measurements made at all experimental points of the P-B plan.

The calculated effects are presented in Table 3 where the significant effects are marked in bold ($\alpha = 0.05$) or underlined bold ($\alpha = 0.01$).

Apart from the main effects of real factors X's represented by coefficients b_1, b_2, …b_6 also coefficients b_0 and the effects b_7 of a dummy factor X_7 as well as the responses, Y_0, measured at the center of the P-B plan are presented in Table 3. They will be discussed later.

GROUPING OF SOLVENTS

Graphs of the scaled effects of factors

To classify solvents into groups exhibiting similar properties, the calculated effects b_u (u = 1, 2, …6) were scaled and presented on graphs where the signal varies in the range from -1 to 1. On the basis of similar patterns of scaled coefficients a preliminary classification of tested solvents was done. The corresponding graphs are shown in Figure 2, where all solvents are included (a) as well as five extracted groups of solvents which exhibited similar properties (similar patterns as seen in graphs, b-f). The similar approach was applied by other authors e.g., to distinguish between different brands of orange juices on the basis of the sensor array measurements (20).

As a reference tool for grouping, chemometric methods were used: cluster analysis and principal component analysis. In the calculations effects of factors, represented by coefficients b_u (u = 1, 2, …, 6), served as variables describing tested solvents.

Cluster analysis[3]

Cluster analysis includes a number of different algorithms and methods for grouping objects into particular categories. It is an exploratory data analysis tool which segregates different objects into groups in a way, that the degree of association between the two objects is maximal if they belong to the same group and minimal otherwise. Therefore, cluster analysis can be exploited to discover structures in data without explaining why they exist (21). For tested solvents, joining (tree clustering) and k-means clustering techniques were used. Results of cluster analysis with the use of Ward's segregation method and on the basis of squared Euclidean distance metric are presented in Figure 3.

In k-means clustering method, the number of five clusters was declared before calculations. This number gave clusters of similar segregation of solvents as the previous methods did. Components of clusters and the Euclidean distances of solvents from the center of a group are presented in Table 4.

[3] Chemometric calculations were carried out using Statistica

Table 3. Effects of factors.

Analyte	Effects								Y_0
	b_0	b_1	b_2	b_3	b_4	b_5	b_6	b_7	
Acetone	**1.139**	**0.180**	–0.016	**0.485**	**–0.068**	**–0.190**	**0.443**	**0.087**	1.146
Acetonitrile	**1.016**	**0.047**	–0.005	**0.379**	**–0.038**	**–0.107**	**0.218**	–0.014	0.852
Benzene	**0.919**	**0.518**	–0.092	–0.082	0.078	**–0.111**	**0.132**	0.093	1.024
Chloroform	**1.495**	**0.227**	**–0.111**	**0.818**	–0.085	**–0.451**	**0.819**	0.076	1.273
Cyclohexane	**0.887**	**0.459**	**–0.083**	**–0.065**	**0.065**	**–0.067**	**0.156**	**0.071**	1.103
1.4-Dioxane	**0.893**	**0.501**	**–0.081**	**–0.096**	**0.083**	**–0.074**	**0.138**	**0.067**	1.066
Ethanol	**0.918**	**0.552**	–0.004	**–0.122**	**0.090**	**–0.148**	0.010	**0.106**	0.882
Ethyl acetate	**1.155**	**0.133**	–0.025	**0.691**	–0.063	**–0.305**	**0.471**	**0.101**	0.836
n-Hexane	**1.120**	0.018	–0.004	**0.715**	0.021	**–0.275**	**0.456**	**0.036**	0.781
n-Heptane	**0.918**	**0.553**	0.006	**–0.123**	**0.088**	**–0.149**	0.001	**0.108**	0.875
Isobutanol	**0.929**	**0.580**	0.013	**–0.132**	**0.091**	**–0.134**	–0.001	**0.096**	0.911
Isopropanol	**1.206**	**0.240**	**–0.101**	**0.627**	**–0.151**	**–0.292**	**0.562**	**0.133**	1.147
Methanol	**1.218**	**0.134**	**–0.054**	**0.727**	**–0.085**	**–0.354**	**0.584**	**0.103**	0.956
Methylene chloride	**0.873**	**0.091**	–0.023	**0.522**	–0.022	**–0.087**	**0.207**	**0.047**	0.629
Methyl ethyl ketone	**1.131**	**0.211**	**–0.050**	**0.411**	**–0.058**	**–0.158**	**0.457**	**0.081**	1.270
Methyl isobutyl ketone	**0.977**	**0.303**	**–0.108**	**0.139**	0.021	–0.003	**0.357**	0.002	1.424
n-Butanol	**1.045**	**0.319**	**–0.089**	**0.187**	-0.017	–0.010	**0.365**	0.015	1.434
n-Propanol	**1.164**	**0.164**	–0.074	**0.694**	**-0.115**	**–0.309**	**0.520**	**0.124**	0.942
Tetrahydrofuran	**1.065**	**0.225**	**–0.038**	**0.366**	**-0.059**	**–0.112**	**0.400**	**0.051**	1.255
Toluene	**0.907**	**0.532**	**–0.075**	**–0.104**	**0.085**	**–0.096**	**0.117**	**0.080**	1.021

Table 4. K-means clustering results.

Cluster 1		Cluster 2		Cluster 3		Cluster 4		Cluster 5	
Component	Distance	Component	Distance	Component	Distance	Component	Distance	Component	Distance
Ethyl acetate	0.138503	Acetone	0.316155	1.4-Dioxane	0.279565	Isobutanol	0.383809	Benzene	0.478989
Methyl isobutyl ketone	0.138503	Acetonitrile	0.404884	Ethanol	0.279565	Isopropanol	0.361374	Chloroform	0.397204
		Methanol	0.365061			n-Butanol	0.587585	Cyclohexane	0.450466
		Methyl ethyl ketone	0.353638			n-Propanol	0.267314	n-Hexane	0.547762
		Tetrahydrofuran	0.250489					n-Heptane	0.608483
								Methylene chloride	0.463691
								Toluene	0.306459

		Clusters				
		1	**2**	**3**	**4**	**5**
Clusters	**1**	0	0.988997	1.524320	1.557611	1.079133
	2	0.988997	0	0.706715	1.167536	1.342875
	3	1.524320	0.706715	0	0.940898	1.757435
	4	1.557611	1.167536	0.940898	0	2.058471
	5	1.079133	1.342875	1.757435	2.058471	0

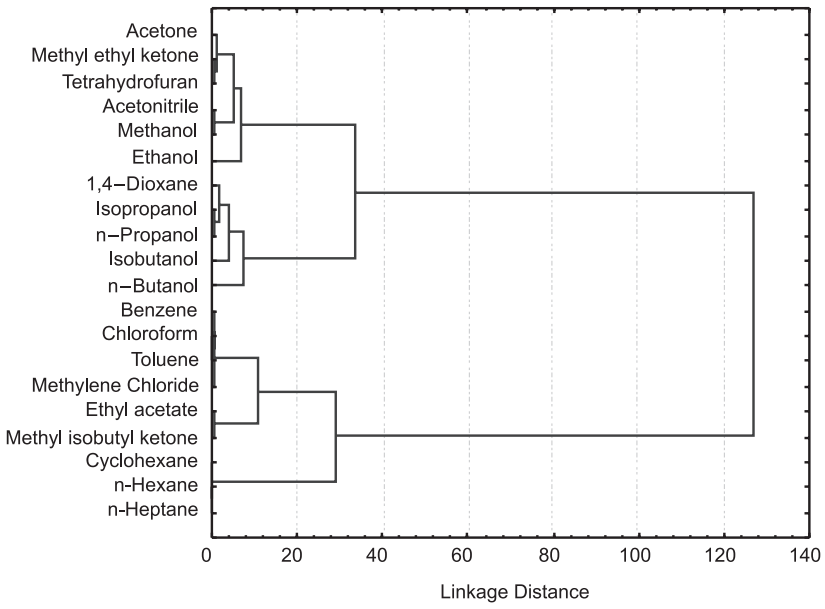

Figure 3. Dendrogram for analyzed solvents

Obtained distances inside groups are significantly shorter than distances between centers of the groups (see Table 4 bottom).

Principal component analysis

Principal component analysis is a chemometric method which reduces data dimension by conversion of the input space into space defined by principal components (PCs). They are ordered by the amount of variance they explain. Usually, it is satisfactory to use two or three principal components in order to transfer the majority of the variation included in the input data. The percent of total variance in the data which is explained by six PC's is presented in Table 5, and selected projections of solvents onto 2D and 3D space of the most important PC's are presented in Figure 4 and the observed structures are indicated.

Selection of significant factors

PCA was used also to select the most important experimental factors impacting quality of HS extraction process and responsible for discrimination between solvents. In Table 6 normalized contributions of coefficients $b_{iu,}$ in the first 3 PCs are presented.

Additionally, in order to visualize significance of individual factors, their effects, b_{iu} have been presented in Figure 5 as a percent of influence from all tested factors.

DISCUSSION

The main purpose of the present investigations was to divide twenty organic solvents into distinct groups, the solvents belonging to a group responding to changes in HS experimental factors similarly. This is important, as optimization of HS conditions can be performed simultaneously for all solvents belonging to the same group, even using a representative solvent for a group. On the other hand, it was also important to select experimental factors which significantly influence efficiency of HS extraction. All the above tasks were realized starting from the experiments carried out according to the Plackett-Burman and then the results were subjected to further handling with use of chemometric tools (CA and PCA). Also application of data visualization methods appeared useful. Because the applied methods gave even more interesting information about the relationship between effectiveness of HS extraction and HS experimental conditions, now let us complete the results presented in the preceding paragraphs with some comment.

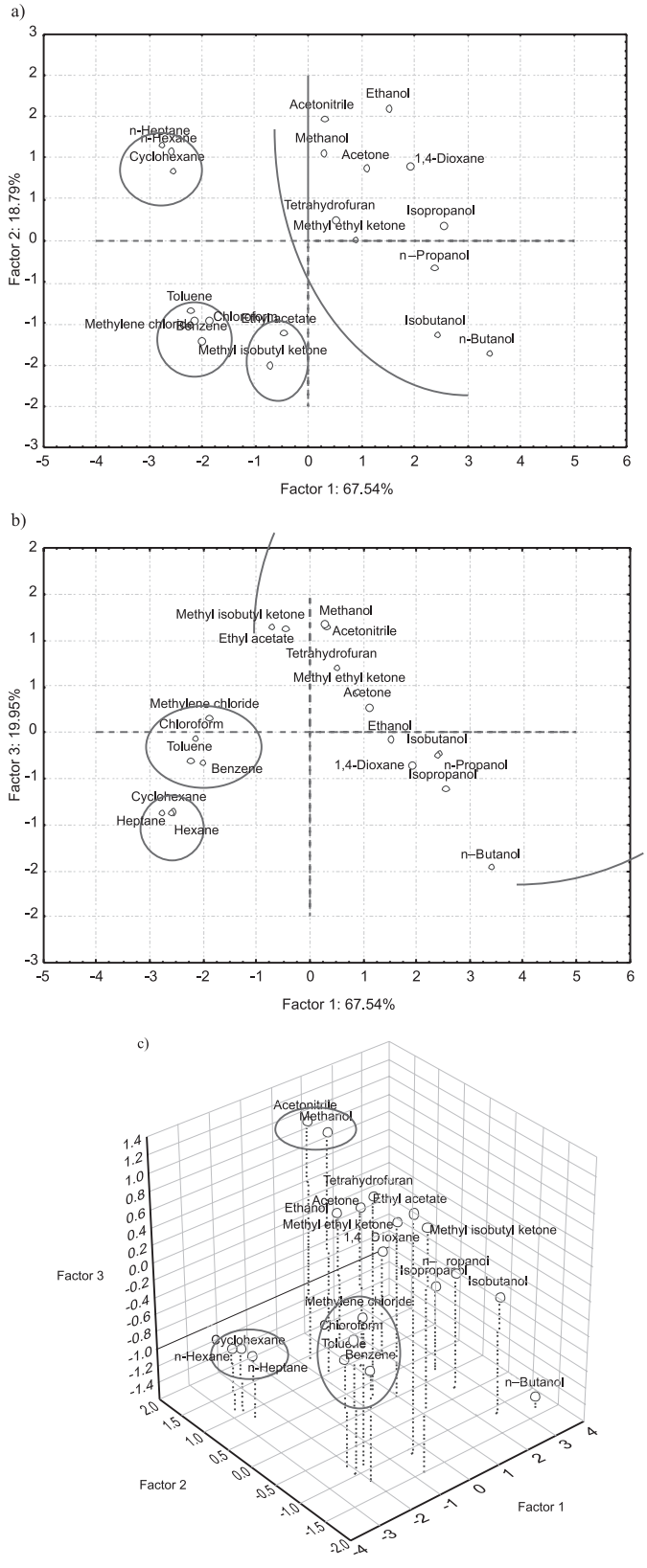

Figure 4. Projections of solvents onto PC1, PC2 (a) and PC1, PC3 (b) planes and 3D space of PC1, PC2, PC3

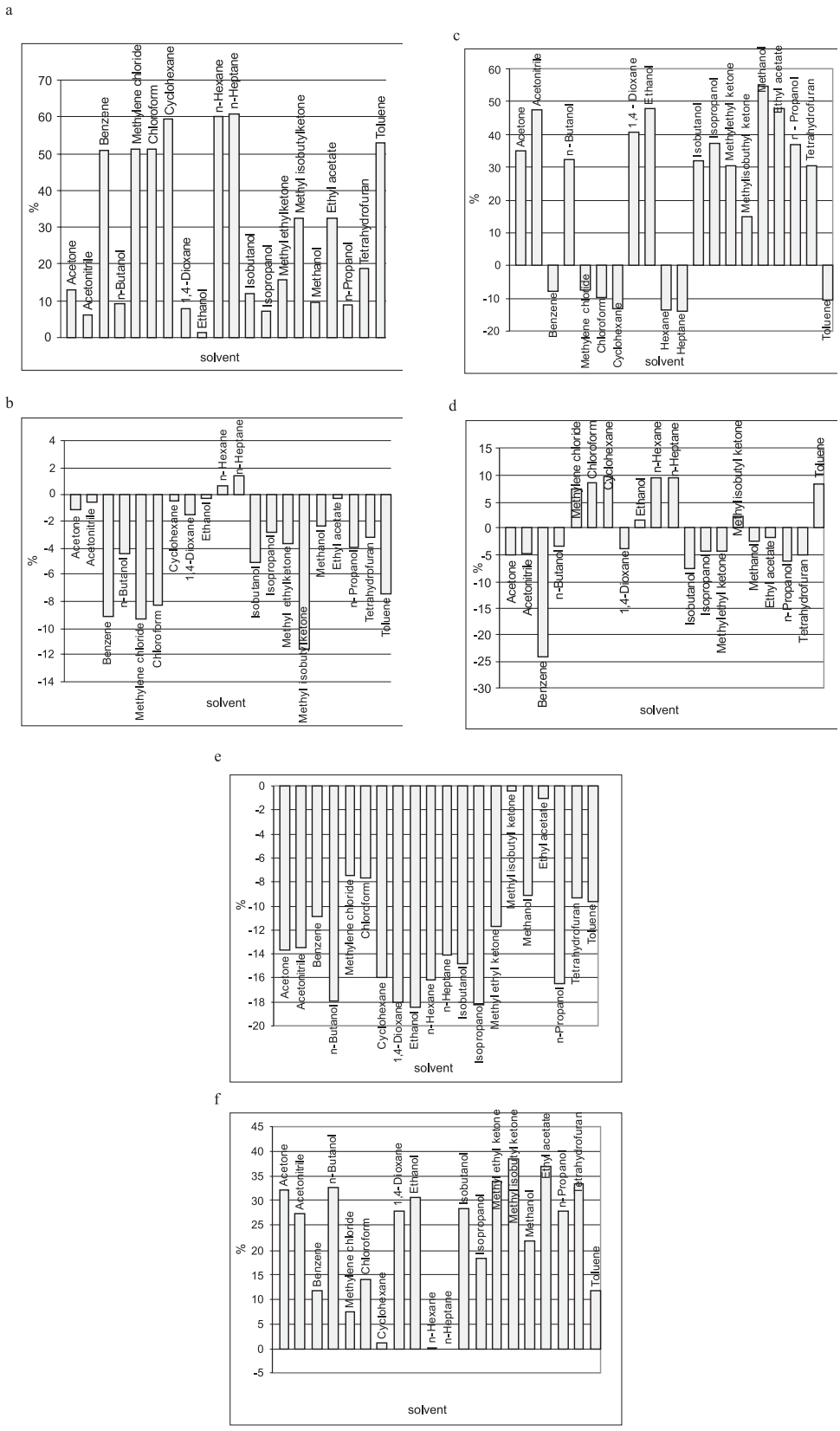

Figure 5. Importance of the effects of six factors presented in percents (see text) for tested solvents a) X_1 – sample volume, b) X_2 – equilibration time, c) X_3 – equilibration temperature, d) X_4 – shaking, e) X_5 – matrix addition, f) X_6 – NaCl addition

Table 5. Percentage variance of principal components.

PC	Eingenvalue	% Variance	Cumulative eigenvalue	% Cumulative variance
PC1	4.052304	67.53839	4.052304	67.5384
PC2	1.127504	18.79174	5.179808	86.3301
PC3	0.596775	9.94624	5.776583	96.2764
PC4	0.159189	2.65315	5.935772	98.9295
PC5	0.055440	0.92401	5.991212	99.8535
PC6	0.008788	0.14646	6.000000	100.0000

Table 6. Variable contributions into the first 3 PCs based on correlations.

Variables	PC1	PC2	PC3
b_1	0.174880	0.083745	0.272761
b_2	0.010836	0.833672	0.007143
b_3	0.237929	0.018856	0.001436
b_4	0.211954	0.002529	0.037622
b_5	0.142533	0.009669	0.678440
b_6	0.221867	0.051530	0.002599

The "influence" (effect b_{i7}) of a dummy factor X_7 in the P-B plan informs (Table 3), in a way, about adequacy of a model Y_i at the experimental points of the plan. Actually, the term $b_{i7}X_7$ makes a "rest" for model Y_i (see eq. 3). It is seen in Table 3 that the above term appeared significant for most solvents tested. Also it is worthwhile to mention differences between b_{i0} and Y_{i0} (difference between responses predicted by model Y_i and determined experimentally at the central point of the P-B plan, respectively) which were found to be significant, especially for ethyl acetate, methylene chloride, methyl ethyl ketone, methyl isobuthyl ketone and tetrahydrofuran. From the above observations it may be concluded that significant interaction effects between factors, as well as nonlinearity of the dependence of responses Y_i on factors X's exist which cannot be directly detected from the results obtained with the use of P-B plan.

To find the factors (their effects) which mostly contribute to discrimination between solvents, their contribution to the PCs was considered (Table 6). It appeared that temperature (X_3), inorganic salt addition (X_6), shaking (X_4) and volume of sample solution (X_1) are the most important components of PC1 which explains 67.5% of total variance in the data. Equilibration time (X_2) has the greatest contribution to PC2 which explains 18.8% of variance. The PC3 explains only 10.0% of variance and is correlated mostly with matrix addition (X_5). These results suggest that matrix addition is a factor which poorly differentiates the analytes.

The significance of factors for all solvents was expressed also in percents (counted separately for every solvent) presented in Figure 5. E.g., it is seen from the figure that following factors have the greatest impact on headspace sensitivity: X_1 (61.0% for heptane), X_3 (54.8% for methanol) and X_6 (38.3% for methyl isobutyl ketone). Matrix addition (X_5) has a great impact on signal area, however, this factor changes signals only one-way (negative) and on similar level for most solvents tested. It is evident from Figure 5e that the presence of matrix is highly undesired as it lowers the response significantly. This observation is compatible with PCA results, and shows that the factor is not useful for grouping the solvents. Salt addition (X_6) also changes the signal one-way, but in desirable direction. Then, the factor is not very useful for differentiation between solvents but it is important for future optimization. Shaking (factor X_4), unlike matrix addition (X_5), presents differentiated impact on solvents signals,

but its influence on the response function (Y) is scarce. To sum up, the following factors can be recommended as significant in optimization of conditions of headspace extraction process: sample solution volume (X_1), equilibration time (X_2), equilibration temperature (X_3) and salt addition (X_6). Also it is worthwhile to notice that effects of factors inform about sensitivity of the method response to the changes of experimental conditions (ruggedness of the method).

On the basis of presented classifying methods (Figs. 2-4) the tested solvents were divided into five groups. The most visible and consistent group create n-hexane, n-heptane and cyclohexane (group I). The second group (group II) is formed by benzene, toluene, chloroform and methylene chloride and was identified by all applied classifying methods. Ethyl acetate and methyl isobutyl ketone form the third observed fraction (group III). Group IV formed by acetone, acetonitrile, ethanol, methanol, methyl ethyl ketone and tetrahydrofuran is visible on scaled responses diagram and on the dendrogram presented in Figure 3. The last educed fraction (group V) consists of 1,4-dioxane, n-propanol, isopropanol, n-butanol and isobutanol.

Figure 3 exhibits still another interesting data structure. There are two groups of solvents which are definitely separated from each other. One of them (the upper one) consists of solvents which do not contain oxygen in their molecules, whereas in the second group there are only two solvents containing oxygen (ethyl acetate and methyl isobutyl ketone) and they form very distinct subgroup.

Although our investigations concerned trace solvents which may be presented in pharmaceutical products, the obtained classification of solvents agrees partly with the segregation proposed by Snyder (22) for liquid chromatography separations. According to this conception, solvents are considered according to their donor, acceptor or donor-acceptor properties and on the basis of experimentally obtained values, and are divided into eight groups. When compared with the results described in the present paper, most of the solvents from group IV and V belong to group II according to Snyder classification (donor-acceptor solvents), solvents classified in our group I belong to group VII and VIII in accordance with Snyder (donor solvents), and finally solvents from the present group III are in group VIa in Snyder classification. It means that Snyder conception dedicated to eluent selection in liquid chromatography may be also helpful as a general indication in determination of HS conditions in gas chromatography, at least at the initial step of investigations. However, as it is presented in this paper, the applied empirical approach based on experimental plans gives more precise results because several factors are involved not only polarity of tested solvents.

CONCLUSIONS

Twenty solvents most frequently used in production of pharmaceuticals, referred as residual solvents, were clustered into five groups, the solvents belonging to a group respond similarly to changes of experimental factors describing conditions of HS extraction in HS-GC determination of solvents. It is why the HS conditions can be optimized for all solvents belonging to a group simultaneously even using a single representative of the group. Moreover, four experimental factors were selected which are most important in the optimization, at least in the starting conditions. In solving the above problems, experiments were carried out according to the Plackett-Burman factorial design, and the resulted main effects of factors served as a basis for application of some visualization approaches and chemometric methods which appeared very useful and effective. As far as we know, it was the first time that the effects of factors were successfully used as variables in CA and PCA as well as in visualization methods applied. It may be said that owing to the chemometric methods applied most useful information has been extracted from the results of experiments carried out according to the Plackett-Burman factorial design. One can presume that involving of the second (and higher) order interactions (effects) between factors, together with the main effects, would still improve the results obtained in our investigations presented above. Then, however, application of another design of experiment would be necessary (e.g., 2^n factorial or fractional factorial, 2^{n-p}) which needs performing much more experiments as compared with what requires P-B design. As usual, a compromise between information gain and work and time consumption is necessary.

REFERENCES

1. ICH Harmonized Tripartite Guideline Q3C (R3): Impurities: Residual solvents http://www.emea. europa.eu/pdfs/human/ich/028395en.pdf.
2. Grodowska K., Parczewski A.: Acta Pol. Pharm. Drug Res 67, 3 (2010).
3. XXXI USP, <467> General Chapter, Organic Volatile Impurities.

4. European Pharmacopoeia 6th edn., 2.4.24. Identification and control of residual solvents.
5. Grodowska K., Parczewski A.: Acta Pol. Pharm. Drug Res 67, 13 (2010).
6. Zhong L., Yieng-Hau H., Gregory P.M. J.: Pharm. Biomed. Anal. 28, 637 (2002).
7. Coran S.A., Giannellini V., Furlanetto S., Bambagitti-Alberti M., Pinazuti S.: J. Chromatogr. A 915, 209 (2001).
8. Camarasu C.C.: J. Pharm. Biomed. Anal. 23, 197 (2000).
9. Dennis K.J., Josephs P.A., Dokladalova J.: Phamacopeial Forum 18, 2964 (1992).
10. Kumar N., Gow J.G.: J. Chromatogr. A 667, 235 (1994).
11. Brillante S.M., Firor R.L., Wylie P.L., Chang I.L., DiUbado D.: Hewlett-Packard application note 228-309, publication no. 5963 – 7109E (1995).
12. George R.B., Wright P.D.: Anal. Chem. 69, 2221 (1997).
13. Natishan T.K., Wu Y.: J. Chromatogr. A 800, 275 (1998).
14. Naddaf A., Balla J.: Chromatographia (Suppl.) 51, 241 (2000).
15. Michulec M., Wardencki W.: Chromatographia 60, 273 (2004).
16. Klaffenbath P., Bruse C., Coors C., Krokenfeld D., Schulz H. G.: LC-GC 15, 1052 (1997).
17. Kumar N., Egoville J.C.: J. Chromatogr. A 859, 113 (1999).
18. Chang C.D., Boguslavskaya M.: Am. Biotechnol. Lab. 16, 11 (1998).
19. Urakami K., Higashi A., Umemoto K., Godo M.: J. Chromatogr. A 1057, 203 (2004).
20. Ciosek P., Sobański T., Augustyniak E., Wróblewski W.: Meas. Sci. Technol. 17, 6 (2006).
21. Neri III M.J.P.: Information Technology Education Policy Framework for Developing Countries: Survey and Cluster Analysis of Worldwide Patterns in Information Technology Education, http://proceedings.informinscience.org/ InSITE2009/InSITE09p035-053Neri685.pdf.
22. Snyder L.R., Kirkland J.J., Dolan J.W. in Introduction to Modern Liquid Chromatography, Wiley, New York 2010.

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