
ANALYSIS

SIMPLEX OPTIMIZATION OF HEADSPACE FACTORS FOR HEADSPACE GAS CHROMATOGRAPHY DETERMINATION OF RESIDUAL SOLVENTS IN PHARMACEUTICAL PRODUCTSKATARZYNA GRODOWSKA^{1,2*} and ANDRZEJ PARCZEWSKI^{2,3}¹Teva Operations Poland, Mogilska 80, 31-546 Kraków, Poland²Jagiellonian University, Faculty of Chemistry, Ingardena 3, Kraków, Poland³Institute of Forensic Research, Westerplatte 9, Kraków, Poland

Abstract: The purpose of the present work was to find optimum conditions of headspace gas chromatography (HS-GC) determination of residual solvents which usually appear in pharmaceutical products. Two groups of solvents were taken into account in the present examination. Group I consisted of isopropanol, n-propanol, isobutanol, n-butanol and 1,4-dioxane and group II included cyclohexane, n-hexane and n-heptane. The members of the groups were selected in previous investigations in which experimental design and chemometric methods were applied. Four factors were taken into consideration in optimization which describe HS conditions: sample volume, equilibration time, equilibrium temperature and NaCl concentration in a sample. The relative GC peak area served as an optimization criterion which was considered separately for each analyte. Sequential variable size simplex optimization strategy was used and the progress of optimization was traced and visualized in various ways simultaneously. The optimum HS conditions appeared different for the groups of solvents tested, which proves that influence of experimental conditions (factors) depends on analyte properties. The optimization resulted in significant signal increase (from seven to fifteen times).

Keywords: residual solvents, headspace-gas chromatography, optimization, simplex method

The impurities which may occur in pharmaceutical products can be assigned to one of the following classes: organic impurities, inorganic impurities and/or residual solvents. Residual solvents 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” (1). They are not desirable in the final product because of their toxicological properties and their taste and smell may be unpleasant for patients. When organic solvents are used in production process, the product should be tested in order to find if residual solvents are present in it and in what concentrations. Acceptable limits of concentrations for the most frequently used organic solvents are included in Q3C ICH guideline (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use). Technique which is widely used in determination of residual solvents

in pharmaceutical products is headspace gas chromatography (HS-GC) (2-6).

The headspace analysis begins with addition of a precise volume or weight of the tested sample into a glass vial, closed and sealed in the next step. This forms two separate phases: a sample phase and a headspace or gaseous phase. The vial is equilibrated in a specified temperature for a specified time. During equilibration, volatile components of the sample (solid or liquid) are gradually extracted into the headspace phase. When the equilibrium is reached and chemical potentials of all sample components are equal in both phases, an aliquot of the headspace is taken and delivered to gas chromatograph system for separation and detection. The dependence between signal measured and concentration of analyte in the headspace phase can be expressed by the following formulas (1) (7):

$$A_i = \text{constant} \times C_{iG} = \text{constant} \times \frac{C_{i0}}{K_i + \beta} \quad (1)$$

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$$\text{where: } K_i = \frac{C_{iS}}{C_{iG}}, \beta = \frac{V_G}{V_S}, K_i \approx \frac{I}{p_i^0 \cdot \gamma_i},$$

$$\log K_i = \frac{B'_i}{T} - C'_i$$

A_i – peak area corresponding to analyte “i”, C_{i0} – original concentration of analyte “i” in the sample, C_{iG} – concentration of analyte “i” in gas phase of the headspace after equilibration, C_{iS} – concentration of analyte “i” in the sample phase after equilibration, K_i – partition (distribution) coefficient of analyte “i”, β – phase ratio, V_G – volume of the gas phase, V_S – volume of the sample phase, p_i^0 – vapor pressure of analyte “i”, γ_i – activity coefficient of analyte “i”, *constant* – incorporates the influence of a number of analytical parameters and the detector response factor, T – temperature, B'_i , C'_i – substance specific constants.

As it is presented in equation (1), concentration of an analyte in HS phase depends on two parameters: partition coefficient (K) and phase ratio (β), but in the end it is related to several other parameters, like: temperature (T), analyte vapor pressure (p_i^0) and activity coefficient (γ_i). Depending on analyte, the above parameters have different influence on signal sensitivity. For example, when for a solvent parameter K is much greater than β , then the headspace sensitivity depends directly on K and phase ratio (sample volume) has practically no influence. Since K changes significantly with temperature and activity coefficient, the effect of temperature or inorganic salt addition (only in cases when water is a sampling medium) on headspace sensitivity may be considerable. On the other hand, when for a solvent parameter K is smaller than β , the signal value is primarily influenced by phase ratio. It means that in such cases temperature has almost no influence on signal sensitivity. Also other sample properties and circumstances may modify efficiency of the headspace process. It is why optimum headspace extraction conditions have to be searched for experimentally. To this end, the variable size simplex optimization method was applied.

Outline of simplex optimization method applied

In experimental optimization it is important that the object under investigation is well defined. Moreover, a criterion function (a response) Y , has to be chosen as well as factors, X_1, X_2, \dots, X_n , which describe experimental conditions in which response Y is determined. The purpose of optimization is to find experimental conditions, i.e., specific values of factors X 's, in which response Y assumes the most desirable value. One of the most efficient strategy of

finding optimum experimental conditions is the sequential simplex optimization (8-12).

The factors X_1, X_2, \dots, X_n make an n -dimensional factorial space in which a simplex is a convex polygon defined by its $n + 1$ vertices. E.g., in two-dimensional factorial space ($n = 2$) it is a triangle and in three-dimensional space ($n = 3$) it is a tetrahedron. The coordinates of the simplex vertices define experimental conditions in which experiments are carried out and the corresponding responses Y are determined.

The optimization is started with determination of responses Y at the vertices of a starting simplex which is build up using coordinates of the “standard simplex”, after starting experimental conditions and the factors' steps, ΔX_i ($i = 1, 2, \dots, n$), have been selected. The vertex at which the worst (least desirable) response was obtained, $Y^{(w)}$, is reflected in the “center of gravity” of the remaining vertices of the simplex. In this way new experimental point, Z , is obtained which together with the remaining vertices (after rejecting the worst vertex) of the preceding simplex makes a new simplex. Depending on the value of response $Y^{(Z)}$ obtained at the new vertex, the following steps are made: reflection, expansion or contraction.

The coordinates $X_1^{(Z)}, X_2^{(Z)}, X_n^{(Z)}$, of the new vertex, Z , are calculated from the following formula (2) (9):

$$X_i^{(Z)} = (1 - \alpha) \times X_i^{(w)} + \frac{\alpha}{n} \times \sum_{j \neq w} X_i^{(j)} \quad (2)$$

where $X_i^{(w)}$ is the coordinate “i” of the worst vertex of the preceding simplex, the sum covers all vertices of the preceding simplex except vertex “w”, and α is a reflection parameter: $\alpha = 2$ gives symmetrical reflection of vertex “w”, $\alpha > 2$ means expansion and $\alpha < 2$ means contraction steps, respectively.

It is important to trace and visualize the progress of simplex optimization (9). This helps also in making decision when to stop optimization. The appropriate approaches were used in the present investigation and are presented below.

EXPERIMENTAL

The optimization was performed for two groups of solvents separately:

Group I: isopropanol, n-propanol, isobutanol, n-butanol and 1,4-dioxane

Group II: cyclohexane, n-hexane and n-heptane

The solvents belonging to a given group exhibit similar properties and behavior in HS conditions, which was proved by experiments carried out according to the Plackett-Burman factorial design

Table 1. Starting simplex.

| Vertex | Factors | | | |
|--------|---------|-------|-------|-------|
| | X_1 | X_2 | X_3 | X_4 |
| 1 | 1 | 10 | 45 | 0 |
| 2 | 2 | 10 | 45 | 0 |
| 3 | 1.5 | 19 | 45 | 0 |
| 4 | 1.5 | 13 | 53 | 0 |
| 5 | 1.5 | 13 | 47 | 7.9 |
| Step | 1 | 10 | 10 | 10 |

and using chemometric methods: CA (cluster analysis) and PCA (principal components analysis) (13).

Reagents and standard solutions

Standard solutions for both groups of solvents were prepared by injecting appropriate amounts of stock standard solutions (prepared in duplicate) into HS vials filled with about 5 mL of water¹. From each stock standard solution there were prepared three vials of standard solution. Concentrations (mg/mL) of solvents in stock standard solutions were: cyclohexane – 22.5, hexane – 6.6, heptane – 25, isobutanol – 25, isopropanol – 25, n-butanol – 25, n-propanol – 25, and 1,4-dioxane – 2. After dilution, concentrations of solvents in HS vials were 100 times lower than in stock standard solutions and they corresponded to maximum allowable limits in accordance to ICH guideline (1) and pharmacopeias (14, 15), under assumption that 250 mg of sample was transferred into headspace vial.

Isopropanol and n-hexane were of gas chromatography grade (Merck, Damstadt, Germany). n-Heptane, 1,4-dioxane and n-butanol were of spectroscopy grade, (Merck). Isobutanol and cyclohexane were of analytically pure grade (POCH, Gliwice, Poland). n-Propanol was of synthesis grade (Merck). Sodium chloride of analytically pure grade (POCH) was used as a salting out factor.

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: column: DB-624 column (30 m × 0.53 mm × 3 μm) from J&W Scientific Agilent Technologies, column temperature program: 400°C hold 10 min, then up to 70°C at the rate 6°C/min and this temperature was kept for 4 min, then the temperature raised again to 100°C at rate 5°C/min and subsequently to 230°C at rate 20°C/min. Injector temperature: 180°C, detector temperature: 250°C, inlet pressure: 3.68 psi, split ratio: 5 : 1.

HS conditions used for reference measurements 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.

Variable size simplex optimization method

Optimization criterion (response function) – equation (3):

$$Y_i = \frac{A_{i \text{ sample}}}{A_{i \text{ std}}} \quad (3)$$

$A_{i \text{ sample}}$ - peak area corresponding to analyte “i” measured in current conditions,

$A_{i \text{ std}}$ - peak area corresponding to analyte “i” measured in reference conditions.

Reference measurements were done to diminish response changes between experiments performed across several days. For this goal, concentrations of solvents in reference solutions were at their maximum allowed limits (according to ICH guideline Q3C), in assumption that 250 mg of sample was analyzed (mass transferred to headspace vial) and the corresponding HS conditions are presented above.

Factors

The following factors were taken into account in the optimization (13):

¹ Actually, volumes of water and standard added made 5 mL.

X_1 – sample volume [mL]; X_2 – equilibration time [min]; X_3 – equilibration temperature [°C]; X_4 – NaCl concentration [%].

The coordinates of vertices of the starting simplex and the steps of factors assumed at the beginning of optimization are presented in Table 1.

In the experimental conditions expressed by coordinates of the starting simplex, the responses, Y_i , were determined, and the vertex at which the lowest value of Y_i was obtained was reflected in the “center of gravity” of the remaining vertices of the simplex. Vertex “1” appeared to be the “worst” both in case of solvents belonging to group I and group II. Then, the optimization was continued in the way described in the corresponding section above. The progress of optimization is presented in Figures 1-3. There are still other useful ways of tracing progress of optimization (9). For each simplex which appears in optimization the gradient of the response function Y is estimated as follows. Using Y values measured at the vertices of a simplex, the coefficients b in the

following polynomial model are determined: $\hat{Y} = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4$, which is a crude approximation of true relationship between response Y and factors X 's within the current simplex. Then, gradient \hat{Y} is determined: $\text{grad } \hat{Y} = b_1\vec{v}_1 + b_2\vec{v}_2 + b_3\vec{v}_3 + b_4\vec{v}_4$, where \vec{v}_i ($i = 1, 2, 3, 4$) are unit vectors corresponding to the axes of the factorial space. In the optimization process $|b_i|$ ($i = 1, 2, 3, 4$) converge to their terminal values. If the extreme of Y lays inside the experimentally available area of factorial space, then $|b_i|$ ($i = 1, 2, 3, 4$) diminish to zero when simplex reaches optimum of the response function Y . Changes of $\text{grad } \hat{Y}$ throughout the optimization progress can be presented as follows: b_i vs. simplex number, or b_i vs. X_i , or $|\text{grad } \hat{Y}|$ vs. simplex number. Selected examples are presented in Figures 4, 5.

RESULTS AND DISCUSSION

It is seen in Figs. 1 and 2 that optimization of HS experimental conditions was very effective for

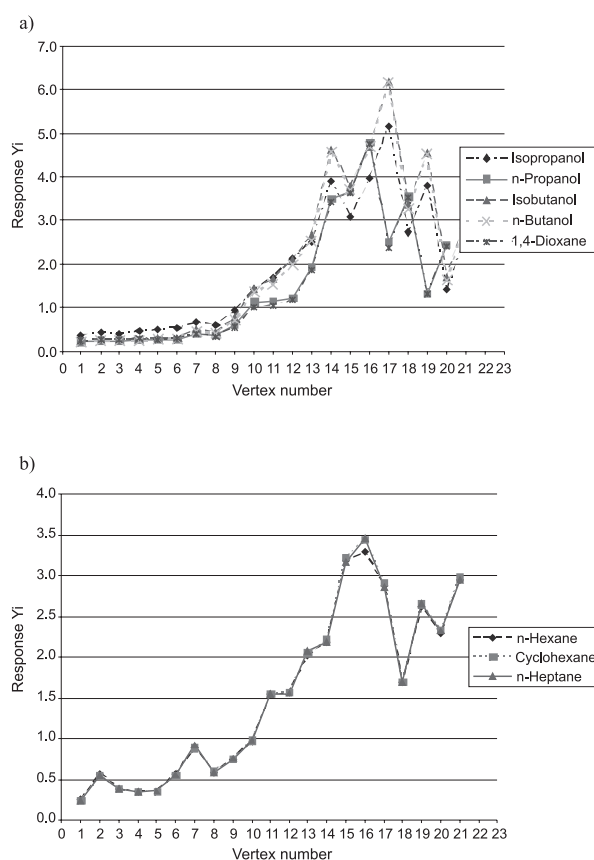


Figure 1. Change of response Y_i in function of vertex number for group I (a) and group II (b) of solvents

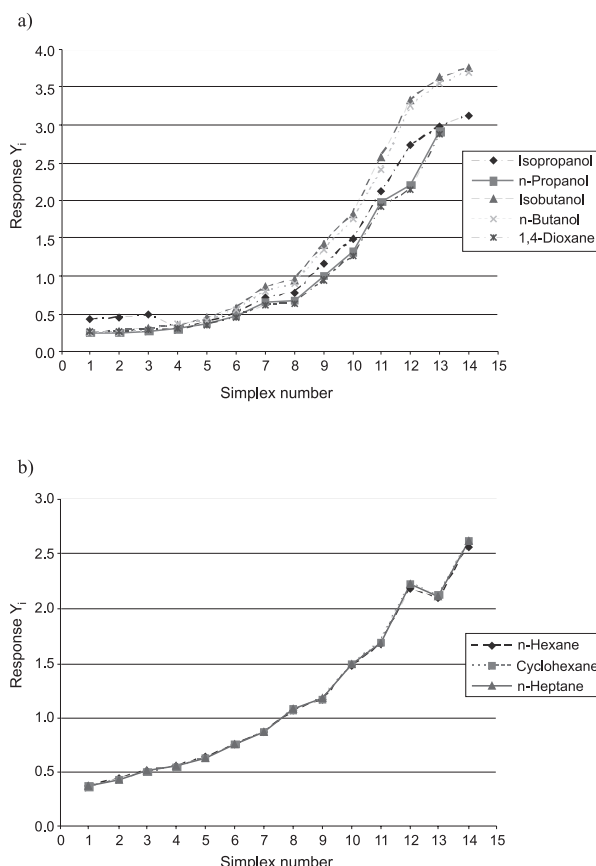


Figure 2. Change of mean response Y_i in function of simplex number for group I (a) and group II (b) solvents

both groups of solvents tested. Actually, the response Y_i (GC relative peak areas) increased ca. fifteen times for solvents belonging to group I and ca. seven times in case of solvents of group II.

Before we comment the results presented in Figures 3-5, it is worthwhile to mention the area of factorial space which was available for experiments in simplex optimization carried out in the present investigation. On the ground of physical barriers like water boiling point, vial volume and NaCl solubility in water, as well as practical aspects like equilibration time longer than one hour, optimization process cannot be proceed without some limitations. Maximum acceptable sample volume was 10 mL. Sixty minutes was a maximum allowed equilibration time and 90°C was maximal equilibration temperature. The last factor, NaCl concentration, could reached only 25% limit. When some factors describing new calculated point (simplex vertex) were outside the assumed limits, they were left at their max-

imum possible level, while other factors were changed on the basis of calculations for this point.

It is evident from Figure 3 that the HS factors changed significantly during optimization and their final values are presented in Table 2. Moreover, it is seen that the factors reached their final values at the border of experimentally available area of factorial space. It is supported by the data exemplified in Figures 4 and 5 by changes of a component b_2 of the response gradient in the course of optimization and it is seen that in the vicinity of the optimum HS conditions the response gradient, $\text{grad} \hat{Y}$, differs from zero. This means that at the optimum HS conditions the response is sensitive to changes in the experimental factors. The fact should be taken into account in the analysis; in order to obtain good precision of determination of the solvents, the HS conditions should be thoroughly maintained.

It is seen in Figure 3 that the optimization paths for solvents belonging to group I and group II differ.

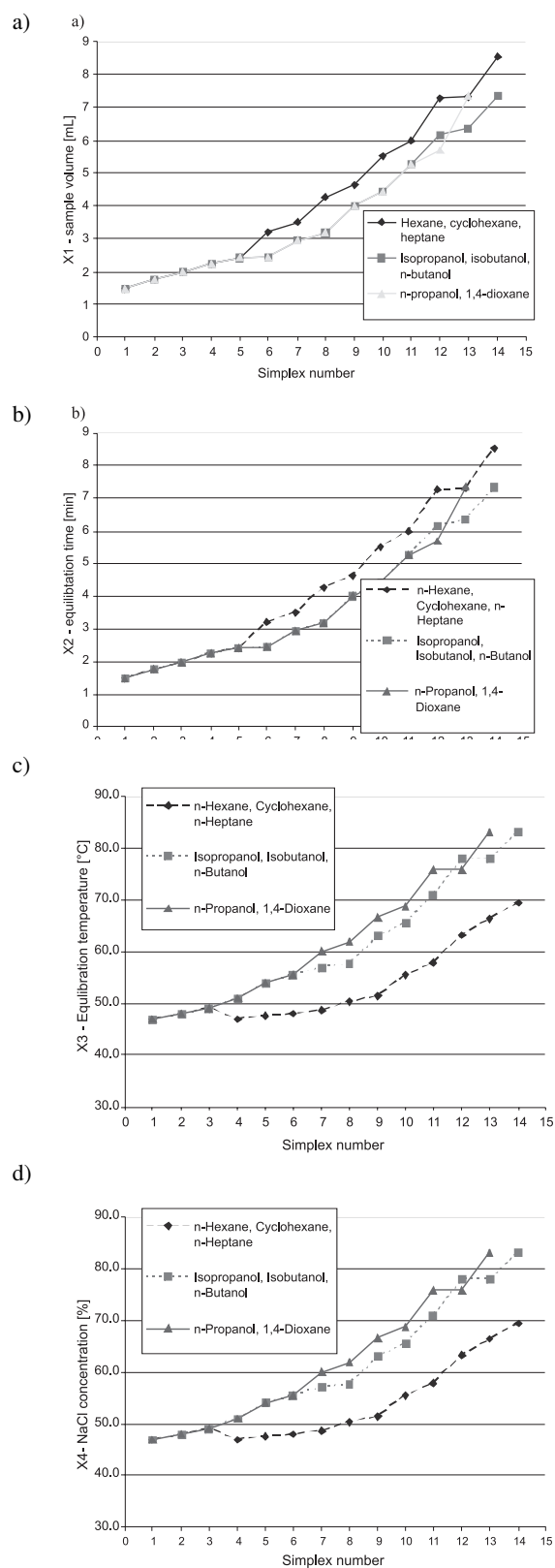


Figure 3. Change of mean values of HS factors (a) – sample volume, (b) – equilibration time, (c) – equilibration temperature, (d) – NaCl concentration vs. simplex number

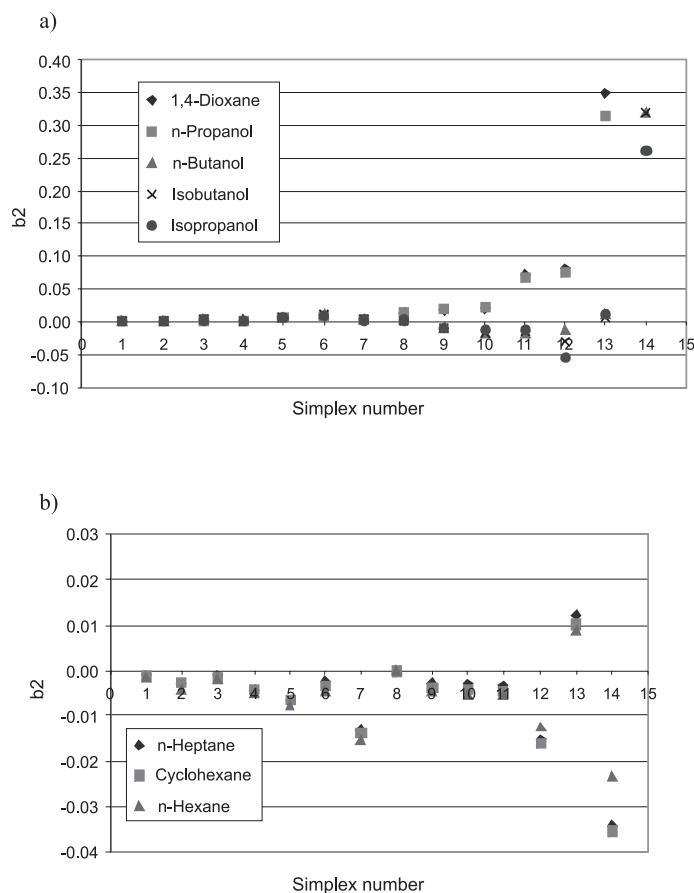


Figure 4. Changes of $\text{grad } \hat{Y}$ component, b_2 , in the course of optimization for group I (a) and group II (b) of solvents

Table 2. Optimal values of factors described by one of vertices from the final simplexes.

| | X_1 [mL] | X_2 [min] | X_3 [°C] | X_4 [%] |
|-------------------------------|------------|-------------|------------|-----------|
| Group I | 9.0 | 60 | 90 | 25 |
| Group II | 9.9 | 51 | 74 | 25 |
| Difference between groups (R) | 0.9 | 9 | 16 | 0 |

It especially concerns factors X_1 , X_3 and X_4 . It means that the solvents belonging to different groups respond the changes of HS experimental parameters differently, which is in agreement with the results previously obtained in grouping of solvents (13).

However, during optimization it turned out that not all solvents belonging to group I behave similarly in particular HS conditions, and for this reason they were analyzed in separate subgroups consisted

of: the first one – isopropanol, isobutanol, n-butanol, and the second one – n-propanol, 1,4-dioxane. At the beginning, they were analyzed together, but after a few experiments, they had to be divided because their next optimization steps were different. In spite of some differences in optimization steps between these subgroups, the final conditions were the same for all solvents belonging to group I. There is no contradiction between this result and the clustering of solvents in group I presented in our preceding

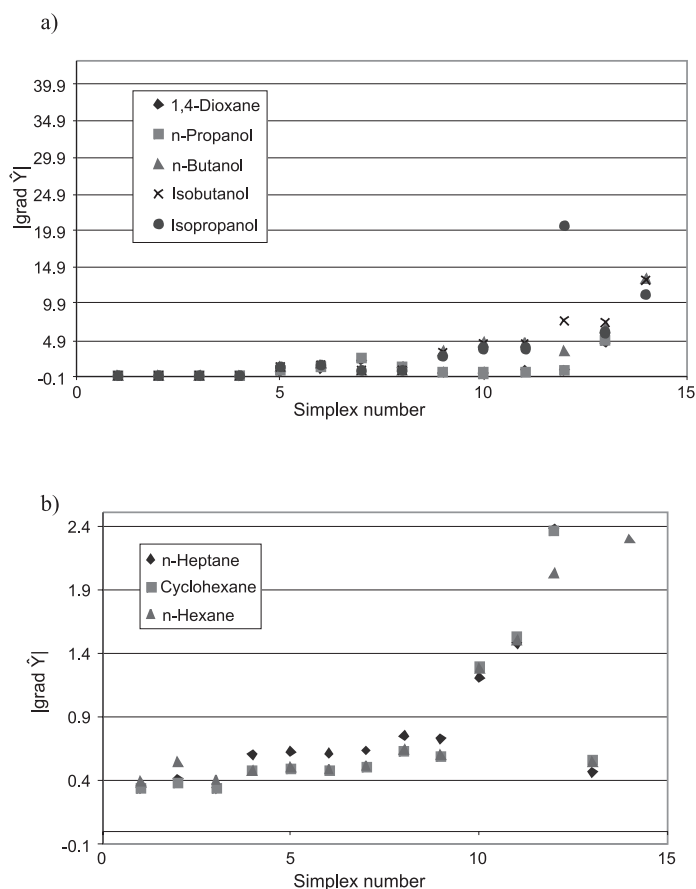


Figure 5. Changes of $\text{grad } \hat{Y}_i$ vs. simplex number assumed in the course of optimization for group I (a) and group II (b) of solvents

paper (13), as the grouping concerned starting conditions of optimization. In the course of optimization process the sensitivity of a response Y_i to experimental factors usually changes, which may result in different behavior of analytes belonging to the previously declared group as it happened in the case discussed. In case of solvents belonging to group II, their properties during optimization appeared rather consistent and they kept together from the beginning to the end of optimization experiments.

Optimization using variable-size simplex method resulted in improvement of signal sensitivity about seven times for group II solvents and from seven to fifteen times for group I solvents. This optimum conditions were described by coordinates of the "optimum" vertex of the final simplex and they are presented in Table 2. For both groups these conditions seem to be similar, but when it comes to the details it is seen that for group II solvents conditions with equilibration temperature greater than 74°C

give worse signal. On the other hand, for group I, solvents optimum is reached in conditions where sample volume is below maximum allowable value (for sample volume it was 10 mL). However, it is inappropriate to conclude about the influence of the single factor, when all used factors have control over obtained signals.

CONCLUSIONS

The applied variable-size simplex optimization proved to be very effective in improving analytical conditions of HS-GC determination of residual solvents. As the result, the signal sensitivity increased by seven to fifteen times for the analyzed solvents. The optimization approach presented in this paper may appear useful in development of new analytical methods for determinations of residual solvents in pharmaceuticals. The obtained results can be used as starting for creation of a database comprising opti-

mum HS conditions of determination of residual solvents in pharmaceuticals which should include still other analytes and procedures in which other than water media are used.

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Received: 22. 05. 2012