Statistical Assessment of Solvent Mixture Models Used for Separation of Biological Active Compounds

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Abstract: Two mathematical models with seven and six parameters have been created for use as methods for identification of the optimum mobile phase in chromatographic separations. A series of chromatographic response functions were proposed and implemented in order to assess and validate the models. The assessment was performed on a set of androstane isomers. Pearson, Spearman, Kendall tau-a,b,c and Goodman-Kruskal correlation coefficients were used in order to identify and to quantify the link and its nature (quantitative, categorical, semi-quantitative, both quantitative and categorical) between experimental values and the values estimated by the mathematical models. The study revealed that the six parameter model is valid and reliable for five chromatographic response factors (retardation factor, retardation factor ordered ascending by the chromatographic peak, resolution of pairs of compound, resolution matrix of successive chromatographic peaks, and quality factor). Furthermore, the model could be used as an instrument in analysis of the quality of experimental data. The results obtained by applying the model with six parameters for deviations of rank sums suggest that the data of the experiment no. 8 are questionable.
Keywords: Mathematical model; chromatographic response functions; statistical assessment; correlation.

Introduction

Chromatographic analysis, defined as the technique used for separation of a mixture of compounds by their distribution between two phases, was invented in 1901 by the Russian botanist Mikhail Semyonovich Tsvet, during his research on plant pigments [1]. He used liquid-adsorption column chromatography with calcium carbonate as adsorbent and petroleum ether/ethanol mixtures as eluent to separate chlorophylls and carotenoids. The method was presented at the XI Congress of Naturalists and Doctors in St. Petersburg in 1901 but the term “chromatography” was used for the first time in 1906 in a paper published in the Berichte der Deutschen Botanischen Gesellschaft Journal [2].

Today, chromatography is a separation method frequently used in chemistry [3,4], biology [5,6], and medicine [7,8] as an analytical technique. The choice of the optimum mobile phase composition is the most difficult and most time-consuming task [9,10]. Optimization procedures have been developed by many researchers in order to obtain the optimum mobile phase. Some geometrical or mathematical models were proposed and assessed [11-15]. Moreover, some optimization methods that use neural networks have been introduced [16,17].

A series of experiments were performed and the optimum mobile phases were obtained for different classes of compounds (steroids [18-20] and benzodiazepines [13,17]). The aim of the present research was to assess through statistical parameters and tests two mathematical models created for optimization of mobile phase in chromatographic separation applied on a set of androstane isomers.

Material and Methods

Experimental Measurements

A set of five previously investigated androstane isomers (5α-androstane-3β-ol, 5α-androstane-3α-ol, 5α-androstane-17β-ol, 5β-androstane-3α,17β-diol, and 5β-androstane-3β,17β-diol) [20] was included into the study. Eleven experimental values were considered (see Table 1). The 11th experiment was a result of an optimization method applied on an objective function that suggests the composition of the optimum mobile phase as 55:19:26 (trichloromethane:propanone:petroleum ether).

The mathematical models presented in Eqs. (1.1) and (1.2) were used in some studies, including in the investigation of the set of androstane isomers presented in [20,21]:

\[
M7(x_1,x_2,x_3) = a_1 x_1 + a_2 x_2 + a_3 x_3 + a_4 x_1 x_2 + a_5 x_1 x_3 + a_6 x_2 x_3 + a_7 x_1 x_2 x_3
\]

(1.1)

\[
M6(x_1,x_2,x_3) = a_1 x_1 + a_2 x_2 + a_3 x_3 + a_4 x_1 x_2 + a_5 x_1 x_3 + a_6 x_2 x_3
\]

(1.2)

where M7, M6 = estimator and predictor of the selected chromatographic parameter; \(x_1, x_2,\) and \(x_3 = \) molar fractions of the three solvents (where \(x_1 + x_2 + x_3 = 1\)); \(a_1, a_2, a_3, a_4, a_5, a_6,\) and \(a_7 = \) model coefficients; first determined based on the best estimation of the selected chromatographic parameter.
Molecules 2008, 13

1619

(Using 7 experiments for M7; 6 experiments for M6) and then used to predict used chromatographic parameter for any composition of the mobile phase (used for not included in model experiments).

The concentration of the solvents into the mixture and the experimental data are presented in Table 1. The chromatography for experiments 1 - 11 was performed on 5×10 cm glass HPTLC plates pre-coated with silica gel 60 F254 (Merck) (Table 1).

Table 1. Experimental data: androstane isomers.

| No. | TCM:Prop:PE* | L1 | l1 | w1 | l2 | w2 | l3 | w3 | l4 | w4 | l5 | w5 |
|-----|--------------|----|----|----|----|----|----|----|----|----|----|----|
| 1   | 33:33:33     | 8.70 | 6.65 | 0.48 | 7.36 | 0.35 | 7.26 | 0.23 | 4.00 | 0.38 | 4.76 | 0.98 |
| 2   | 0:0:100      | 8.83 | 0.00 | 0.42 | 0.00 | 0.44 | 0.00 | 0.22 | 0.00 | 0.25 | 0.00 | 0.21 |
| 3   | 0:100:0      | 8.75 | 8.29 | 0.37 | 8.49 | 0.26 | 8.49 | 0.11 | 7.93 | 0.28 | 7.79 | 0.59 |
| 4   | 100:0:0      | 9.00 | 1.21 | 0.62 | 2.05 | 0.45 | 1.43 | 0.41 | 0.05 | 0.23 | 0.19 | 0.30 |
| 5   | 50:0:50      | 8.93 | 0.54 | 0.56 | 0.98 | 0.38 | 0.68 | 0.27 | 0.00 | 0.26 | 0.00 | 0.25 |
| 6   | 50:50:0      | 8.84 | 6.71 | 0.55 | 7.12 | 0.31 | 7.05 | 0.20 | 5.31 | 0.36 | 5.65 | 0.69 |
| 7   | 0:50:50      | 8.76 | 8.44 | 0.36 | 8.56 | 0.11 | 8.56 | 0.05 | 7.35 | 0.31 | 7.20 | 1.38 |
| 8   | 10:10:80     | 8.86 | 3.49 | 0.60 | 4.71 | 0.42 | 4.51 | 0.28 | 0.53 | 0.27 | 0.64 | 1.41 |
| 9   | 80:10:10     | 8.87 | 5.08 | 0.69 | 6.71 | 0.51 | 6.06 | 0.34 | 1.01 | 0.32 | 2.32 | 0.63 |
| 10  | 10:80:10     | 8.82 | 8.24 | 0.52 | 8.41 | 0.24 | 8.46 | 0.14 | 7.38 | 0.32 | 7.27 | 0.96 |
| 11  | 55:19:26     | 18.95 | 3.43 | 0.82 | 5.86 | 1.16 | 11.52 | 1.43 | 13.44 | 1.25 | 14.38 | 1.32 |

*Trichloromethane (CHCl3 [mL]) : Propanone (CH3COCH3 [mL]) : Petroleum ether ([mL])

L = migration distance of the eluent e

l_i = migration distance of i_th compound in eluent e (where i = 1, 2, ..., 5)

w_i = spot width of i_th compound (where i = 1, 2, ..., 5)

Statistical Validation

The statistical hypothesis of the research was as follows: the mathematical model with seven parameters proposed by Eq (1.1.), and the model with six parameters proposed by Eq (1.2) are reliable and valid models for estimation of a given response function (H0).

A series of parameters were defined and proposed to be used as estimators of the separation quality (see Table 2). The terms and symbols used in the assessment of the mathematical model are as were established by the International Union of Pure and Applied Chemistry (IUPAC) in the Nomenclature for Chromatography [22].

The model is not considered reliable and valid if the null hypothesis (H0) is rejected at a significance level of 5% in the investigation of the response factors (see Table 2). Pearson (r), Spearman (ρ), Kendall (τ-a,b,c) and Gamma (Γ) correlation coefficients [23-25] were used in order to identify and to quantify the nature of the link (quantitative, categorical, semi-quantitative, quantitative and categorical) between experimental and estimated values. The correlation approach was choose for analysis of the quality of the models (Eq (1.1), and Eq (1.2), respectively) due to its ability of identification of linear relationship between two variable (in our case the experimental and estimated values by the proposed mathematical models from Eq (1.1) and Eq (1.2), respectively).
Table 2. Chromatographic response function for statistical assessment.

| Parameter                        | Formula                                                                 | Eq. | Notes                                                                 |
|----------------------------------|--------------------------------------------------------------------------|-----|----------------------------------------------------------------------|
| Retardation factors              | RF(i,e) = l(i,e)/l(e)                                                    | (2) | i separated compound                                                 |
| (RF) matrix                      |                                                                          |     | e the mobile phase                                                   |
|                                  | l(i,e) / l(e)                                                            |     | migration distance of i in e                                          |
|                                  |                                                                          |     | migration distance of e                                              |
| Ordered RF                       | RFO(i,e) = 2(l(i+1,e)-l(i,e))/l(e)                                       | (3) | i^th migration coordinate in the list of migration, ordered by length|
|                                  | l(i,e)                                                                   |     |                                                                      |
| Resolution matrix                | RSM(i,j,e) = 2(l(i,e)-l(j,e))/(w(i,e)+w(j,e))                            | (4) | a separated compound                                                 |
|                                  | w(i,e)                                                                  |     |                                                                      |
|                                  | w(j,e)                                                                  |     |                                                                      |
| Resolution of adjacent spots     | RSO(i,e) = 2(l(i+1,e)-l(i,e))/(w(i+1,e)+w(i,e))                          | (5) | i^th migration coordinate in the list of migration, ordered by length|
| matrix                           | l(i,e)                                                                   |     |                                                                      |
| Number of components             | nc(e) = Σ [l(i+1,e)-l(i,e)>w(i+1,e)+w(i,e)]/8                           | (6) | number of components observed in e                                   |
|                                  | nc(e)                                                                   |     |                                                                      |
| Maximum number of components     | mnc = nc(e)                                                              | (7) | from all experimented mobile phases (or previous knowledge)          |
|                                  |                                                                          |     |                                                                      |
| Retardation factors deviation    | RFD(e) = √(Σ (ΔRF(i,e)-1/mnc)^2)/nc(e)(nc(e)+1)                          | (8) | theoretical difference between two retardation factors              |
|                                  | l(e)                                                                    |     |                                                                      |
|                                  |                                                                          |     |                                                                      |
| Informational energy             | IENE(e) = mc^2 - Σ (ni)^2                                              | (9) | number of compounds that migrate into i^th equidistant interval from mnc |
|                                  |                                                                          |     |                                                                      |
| Informational entropy            | IEnt(e) = Σ(ni)log2(ni)                                                 | (10)|                                                                      |
|                                  |                                                                          |     |                                                                      |
| Resolution sum                   | RSS(e) = Σ_i RSO(i,e)                                                   | (11)| average indicator for separation                                    |
|                                  |                                                                          |     |                                                                      |
| Effective plates number squared  | QN_{eff}(e) = 4·l(e)/(Σ_i w(e,i))                                      | (12)| average indicator for a hypothetic quantitative analysis          |
| root                             |                                                                          |     |                                                                      |
| Resolution divided by the number | RSP(e) = 25·RSS(e)/QN_{eff}(e)                                          | (13)| composite indicator for separation expressed as proportion; note that 4·RSS(e) → QN_{eff}(e) for an ideal separation |
| of effective plates              |                                                                          |     |                                                                      |
| Average resolution for separation| RSA(e) = RSS(e)/nc(e)                                                   | (14)| average indicator for separation                                    |
|                                  |                                                                          |     |                                                                      |
| Relative resolution product      | RRP(e) = Π_i RSO(i,e)/Σ_i RSO(i,e)                                      | (15)| average indicator for separation                                    |
|                                  |                                                                          |     |                                                                      |
| Minkowski type mean of resolutions | RSR(e) = (Σ_i (RSO(i,e))^1/n)^p/nc(e)^p; p = 2                          | (16)| is better descriptor for separation than RSA                        |
|                                  |                                                                          |     |                                                                      |
Table 2. Cont.

| Quality factor | \[ QF(e) = \min_{i,j} RSM(i,j,e) = \min_{i} RSO(i,e) \] | Notes: |
|----------------|-------------------------------------------------|-------|
|                | 最差一个定义分离的分辨率 |       |

Notes:

- Informational energy is a quality factor computed by the Logit method, which is equal with 0 for an ideal separation
- Informational energy is a quality factor which is equal with 0 for an ideal separation
- Part of the entries in Table 2 were previously used in the literature, with different names; thus, relating [20], RFD was reported as Sm.

A significant correlation coefficient (at a significance level of 5%) sustains the validity of the model from Eq (1.1) and Eq (1.2) in the estimation of chromatographic response factors of interest. An online resource hosted by AcademicDirect was used in order to calculate correlation coefficients and associated statistic parameters (http://l.academicdirect.org/Statistics/linear_dependence/).

The difference between two correlation coefficients was tested by using Statistica 6.0 software, Basic Statistics and Tables - Differences tests - Differences between two correlation coefficients.

If Pearson’s correlation coefficient is much smaller than Spearman’s correlation coefficient applied to the same variables, it can be concluded that the variable of interest correlated consistently (if both are statistically significant), but not in a linear quantitative manner (i.e. may not be linear; may not be quantitative).

A linear relationship between experimental and estimated values (given by Eq (1.1) and Eq (1.2), respectively) was considered to be proven when Pearson’s and Spearman’s correlation coefficients had similar values (i.e. there is no significant statistical difference) that are statistically significant different from zero. The Kendall’s as well as Gamma correlation coefficients make no assumption whatsoever about the distribution of the investigated values (it is a ratio that investigate the “concordant” and “discordant” pairs). Opposite to Pearson's correlation coefficient, Kendall’s tau correlation coefficients are measures of correlation between two ordinal-level variables. When Kendall’s tau correlation coefficient are applied, for more concordant than discordant pairs the value of the coefficient is positive, for equal concordant and discordant pairs the coefficient is zero.

Results and Discussion

A series of results were obtained by applying the parameters described in Table 2 on the set of androstane isomers. Four response functions were computed as the measurement space (Eq (2)-Eq (5)): retardation factor, retardation factors ordered ascending by the chromatographic peak, resolution of separation, and resolution of separation of successive peaks. The proposed response functions (Eq (6)-Eq (17)) for investigation of the reliability and validity of the model presented in Eq (1.1) and Eq (1.2) where computed based on the results of Eq (2)-Eq (5).

The retardation factor matrix (the response function of the compound separation in chromatography, defined as the relative migration distance) for each investigated compound is presented in Table 3.
Table 3. Matrix of retardation factor: experimental vs estimated.

| No | Experimental | Estimated by Eq(1.1) | Estimated by Eq(1.2) |
|----|--------------|----------------------|----------------------|
|    | AI1 AI2 AI3 AI4 AI5 | AI1 AI2 AI3 AI4 AI5 | AI1 AI2 AI3 AI4 AI5 |
| 1  | 0.764 0.845 0.834 0.460 0.547 | 0.006 0.021 0.134 0.159 0.228 |
| 2  | 0.000 0.000 0.000 0.000 0.000 |
| 3  | 0.947 0.970 0.970 0.906 0.890 |
| 4  | 0.134 0.228 0.159 0.006 0.021 |
| 5  | 0.060 0.110 0.076 0.000 0.000 |
| 6  | 0.759 0.805 0.798 0.601 0.629 |
| 7  | 0.963 0.977 0.977 0.839 0.822 |
| 8  | 0.394 0.532 0.509 0.060 0.072 0.291 0.314 0.308 0.203 0.216 0.215 0.219 0.271 0.279 0.284 |
| 9  | 0.573 0.756 0.683 0.114 0.262 0.309 0.393 0.347 0.139 0.174 0.157 0.172 0.289 0.317 0.364 |
| 10 | 0.934 0.954 0.959 0.837 0.824 1.017 1.052 1.053 0.878 0.891 0.882 0.903 0.997 1.024 1.022 |
| 11 | 0.181 0.309 0.608 0.709 0.759 0.505 0.591 0.565 0.253 0.324 0.309 0.323 0.438 0.465 0.491 |

AI1 = 5α-androstane-3β ol; AI2 = 5α-androstane-3α ol
AI3 = 5α-androstane-17β ol; AI4 = 5β-androstane-3α,17β diol; AI5 = 5β-androstane-3β,17β diol

The experimental and estimated retardation factor had identical values for first experiments because these were used to construct the model: the coefficients of the model with seven parameters proposed by Eq (1.1) were calculated based on experiments 1...7 presented in Table 1. A similar procedure was followed for experiments 2...7 included in the model with six parameters proposed by Eq (1.2). The differences between experimental and estimated values (for the experiments not included in learning set) showed that the great variation was obtained by the experiment no. 11. The difference varied from -0.324 (AI1 = 5α-androstane-3β ol, experiment no. 11, model M7) to 0.584 (AI4 = 5β-androstane-3α,17β diol, experiment no. 11, model M6). The estimated values were greater than the experimental values in half of the cases. Systematically, the estimated values were greater than experimental values for experiment no. 10 - Eq (1.1) and lower than experimental values for experiment no. 1 - Eq (1.2).

The correlation analysis on experimental versus estimated retardation factor give the results presented in Table 4. The analysis of the values of correlation coefficients (all values are higher than 0.5 and all correlation are statistically significant p < 1.39·10^-2) revealed that the models from Eq (1.1) and Eq (1.2) have good abilities in estimation of chromatographic retardation factor.

Table 4. Correlation analysis on retardation factor: experimental versus estimated.

| Name             | Correlation coefficient | p-value        | Statistical parameter |
|------------------|-------------------------|----------------|----------------------|
| Eq(1.1), n = 20  |                         |                |                      |
| Pearson          | r = 0.7214              | 3.31·10^-4     | tPrs,1 = 4.42        |
| Spearman         | ρ = 0.7789              | 5.19·10^-5     | tSpm,1 = 5.27        |
| Semi-Q           | r_{aq} = 0.7496         | 1.42·10^-4     | tQ = 4.80            |
| Kendall ta       | τ_{ken,a} = 0.6316      | 9.89·10^-5     | ZKen,a = 3.89        |
| Kendall tb       | τ_{ken,b} = 0.6316      | 9.89·10^-5     | ZKen,b = 3.89        |
| Kendall tc       | τ_{ken,c} = 0.6000      | 2.17·10^-4     | ZKen,tc = 3.70       |
| Gamma            | Γ = 0.6316              | 1.39·10^-2     | ZΓ = 2.46            |
The highest correlation coefficient is obtained by the Spearman method and leads to the idea that the retardation factor is a categorical not a quantitative variable. Statistically, there was not identified any significant difference between correlation coefficients obtained by different methods, neither for Eq (1.1) nor for Eq (1.2) (the lowest value of 0.3465 was obtained in comparison of Spearman and Kendall - Eq (1.1); a p-value of 0.0780 was obtained in comparison of Spearman and Kendall τc - Eq (1.2)).

The retardation matrix ordered ascending for each chromatographic peak was obtained based on Eq (3). The results for experimental and estimated determinations are presented in Table 5. The correspondence between each peak and the compound was known on the data presented in Table 5. The difference between experimental and estimated values varied from -0.155 (1st peak, experiment no. 8, Eq (1.2)) to 0.363 (5th peak, experiment no. 9, Eq (1.1)) and 0.675 (4th peak, experiment no. 1, Eq (1.2)). Systematically, the estimated values were higher than experimental values for experiment no. 10, for both Eq (1.1) and Eq (1.2). The correlation analysis between experimental and estimated values (by Eq (1.1) and Eq (1.2), respectively) leads to the results presented in Table 6.

Table 5. Matrix of retardation factors ordered by the chromatographic peak: experimental vs estimated.

| No. | Experimental peak | Estimated peak by Eq(1.1) | Estimated peak by Eq(1.2) |
|-----|-------------------|--------------------------|--------------------------|
|     | 1<sup>st</sup>  | 2<sup>nd</sup> | 3<sup>rd</sup> | 4<sup>th</sup> | 5<sup>th</sup> | 1<sup>st</sup> | 2<sup>nd</sup> | 3<sup>rd</sup> | 4<sup>th</sup> | 5<sup>th</sup> | 1<sup>st</sup> | 2<sup>nd</sup> | 3<sup>rd</sup> | 4<sup>th</sup> | 5<sup>th</sup> |
| 1   | 0.460 | 0.547 | 0.764 | 0.834 | 0.845 | 0.006 | 0.021 | 0.134 | 0.159 | 0.228 |
| 2   | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 3   | 0.890 | 0.906 | 0.947 | 0.970 | 0.970 | 0.006 | 0.021 | 0.134 | 0.159 | 0.228 |
| 4   | 0.006 | 0.021 | 0.134 | 0.159 | 0.228 | 0.006 | 0.021 | 0.134 | 0.159 | 0.228 |
| 5   | 0.000 | 0.000 | 0.060 | 0.076 | 0.110 | 0.060 | 0.076 | 0.110 | 0.060 | 0.076 |
| 6   | 0.601 | 0.629 | 0.759 | 0.798 | 0.805 | 0.601 | 0.629 | 0.759 | 0.798 | 0.805 |
| 7   | 0.822 | 0.839 | 0.963 | 0.977 | 0.977 | 0.822 | 0.839 | 0.963 | 0.977 | 0.977 |
| 8   | 0.060 | 0.072 | 0.394 | 0.509 | 0.532 | 0.060 | 0.072 | 0.394 | 0.509 | 0.532 |
| 9   | 0.114 | 0.262 | 0.573 | 0.683 | 0.756 | 0.114 | 0.262 | 0.573 | 0.683 | 0.756 | 0.114 | 0.262 | 0.573 | 0.683 | 0.756 |
| 10  | 0.824 | 0.837 | 0.934 | 0.954 | 0.959 | 0.824 | 0.837 | 0.934 | 0.954 | 0.959 | 0.824 | 0.837 | 0.934 | 0.954 | 0.959 |
| 11  | 0.181 | 0.309 | 0.608 | 0.709 | 0.759 | 0.181 | 0.309 | 0.608 | 0.709 | 0.759 |

L = migration distance of the eluent
The difference between two correlation coefficients was tested and the results are presented in Table 7. By analyzing the results from Table 7 it can be seen that the investigated chromatographic response function is more like to be a categorical variable but not a rank variable then a quantitative variable (the Spearman rank correlation coefficient is statistically significant greater that Kendall $\tau_c$ applied on Eq (1.1)). The data presented in Table 6 revealed that all correlation coefficient were statistically significant ($p \leq 3.82 \cdot 10^{-5}$). Thus, it can be concluded that the link between experimental and estimated by Eq (1.1) and Eq (1.2) data are linear related and sustain the validity of the models from Eq (1.1) and Eq (1.2) for this chromatographic response function.

Table 6. Correlation analysis on retardation factor ordered ascending by the chromatographic peak (experimental vs estimated values).

| Name       | Correlation coefficient | p-value   | Statistical parameter |
|------------|-------------------------|-----------|-----------------------|
| Eq(1.1), n = 20 |                         |           |                       |
| Pearson    | $r = 0.8654$            | $8.38 \cdot 10^{-7}$ | $t_{Prs,1} = 7.33$ |
| Spearman   | $\rho = 0.9579$         | $3.39 \cdot 10^{-11}$ | $t_{Spn,1} = 14.15$ |
| Semi-Q     | $r_{sq} = 0.9105$       | $2.53 \cdot 10^{-8}$  | $t_{Q} = 9.34$       |
| Kendall ta | $\tau_{Ken,a} = 0.8526$| $1.47 \cdot 10^{-7}$  | $Z_{Ken,a} = 5.26$  |
| Kendall tb | $\tau_{Ken,b} = 0.8526$ | $1.47 \cdot 10^{-7}$  | $Z_{Ken,b} = 5.26$  |
| Kendall tc | $\tau_{Ken,c} = 0.8100$| $5.94 \cdot 10^{-7}$  | $Z_{Ken,c} = 4.99$  |
| Gamma      | $\Gamma = 0.8526$       | $7.42 \cdot 10^{-6}$  | $Z_{\Gamma} = 4.48$  |
| Eq(1.2), n = 25 |                         |           |                       |
| Pearson    | $r = 0.8292$            | $3.02 \cdot 10^{-7}$  | $t_{Prs,1} = 7.11$  |
| Spearman   | $\rho = 0.9008$         | $8.45 \cdot 10^{-10}$ | $t_{Spn,1} = 9.95$  |
| Semi-Q     | $r_{sq} = 0.8642$       | $2.58 \cdot 10^{-8}$  | $t_{Q} = 8.24$      |
| Kendall ta | $\tau_{Ken,a} = 0.7667$| $7.80 \cdot 10^{-8}$  | $Z_{Ken,a} = 5.37$  |
| Kendall tb | $\tau_{Ken,b} = 0.7667$ | $7.80 \cdot 10^{-8}$  | $Z_{Ken,b} = 5.37$  |
| Kendall tc | $\tau_{Ken,c} = 0.7360$| $2.51 \cdot 10^{-7}$  | $Z_{Ken,c} = 5.16$  |
| Gamma      | $\Gamma = 0.7667$       | $3.82 \cdot 10^{-5}$  | $Z_{\Gamma} = 4.12$  |

Table 7. Matrix of p-values: test of difference between two correlation coefficients.

|                        | Pearson  | Spearman | Semi-Q  | Kendall ta | Kendall tb | Kendall tc | Gamma |
|------------------------|----------|----------|---------|------------|------------|------------|-------|
| Eq(1.1), n = 20        |          |          |         |            |            |            |       |
| Pearson                | 1.0000   | 0.3510   | 0.7287  | 0.5805     | 0.5805     | 0.4346     | 0.5805|
| Spearman               | 0.0824   | 1.0000   | 0.5559  | 0.1408     | 0.1408     | 0.0903     | 0.1408|
| Semi-Q                 | 0.5743   | 0.2305   | 1.0000  | 0.3699     | 0.3699     | 0.2614     | 0.3699|
| Kendall ta             | 0.7416   | 0.0519   | 0.2468  | 1.0000     | 0.3699     | 0.2614     | 0.3699|
| Kendall tb             | 0.7416   | 0.0519   | 0.2468  | 1.0000     | 1.0000     | 1.0000     | 0.8178|
| Kendall tc             | 0.3783   | 0.0223   | 0.0890  | 0.5803     | 0.5803     | 1.0000     | 0.8178|
| Gamma                  | 0.7416   | 0.0519   | 0.2468  | 1.0000     | 1.0000     | 0.5803     | 1.0000|

The resolution of separation between any two investigated androstanne isomers could be considered one of the top-three quality measurements of a chromatographic separation. The resolution matrix
between pairs of androstane isomers are presented in Table 8. The highest the resolution value between two compounds, the better the separation is considered.

The difference between experimental and estimated values for experiments 8-11 varied from to -7.232 (the resolution between 5β-androstane-3α,17β-diol and 5β-androstane-3β,17β-diol, experiment no. 8, Eq (1.1)) to 11.827 (the resolution between 5α-androstane-17β-ol and 5β-androstane-3α,17β-dio, experiment no.8, Eq (1.2)). With a single exception, the estimated exceed the experimental values (experiment no. 10, for both Eq (1.1) and Eq (1.2), respectively). The results of the correlation analysis are presented in Table 9.

**Table 8.** Resolution matrix of pairs of compounds: experimental vs estimated.

| No. | A11-A12 | A11-A13 | A11-A14 | A11-A15 | A12-A13 | A12-A14 | A12-A15 | A13-A14 | A13-A15 | A14-A15 |
|-----|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| 1   | 1.687   | 1.718   | 6.163   | 2.589   | 0.310   | 9.178   | 3.895   | 10.689  | 4.132   | 1.118   |
| 2   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   |
| 3   | 0.635   | 0.833   | 1.108   | 1.042   | 0.000   | 2.074   | 1.647   | 2.872   | 2.000   | 0.322   |
| 4   | 1.570   | 0.427   | 2.729   | 2.217   | 1.442   | 5.882   | 4.960   | 4.313   | 3.493   | 0.528   |
| 5   | 0.936   | 0.337   | 1.317   | 1.333   | 0.923   | 3.063   | 3.111   | 2.566   | 2.615   | 0.000   |
| 6   | 0.953   | 0.907   | 3.077   | 1.855   | 0.275   | 5.403   | 3.120   | 6.214   | 3.348   | 0.476   |
| 7   | 0.511   | 0.585   | 3.254   | 1.425   | 0.000   | 5.762   | 1.826   | 6.722   | 1.902   | 0.178   |
| 8   | 2.392   | 2.318   | 6.805   | 2.836   | 0.571   | 12.116  | 4.448   | 14.473  | 4.580   | 0.131   |
| 9   | 2.717   | 1.903   | 8.059   | 4.182   | 1.529   | 13.735  | 7.702   | 15.303  | 7.711   | 2.758   |
| 10  | 0.447   | 0.667   | 2.048   | 1.311   | 0.263   | 3.679   | 1.900   | 4.696   | 2.164   | 0.172   |
| 11  | 2.455   | 7.191   | 9.671   | 10.234  | 4.371   | 6.290   | 6.871   | 1.433   | 2.080   | 0.732   |

|     | Estimated by Eq(1.1) |       |       |       |       |       |       |       |       |       |
|-----|-----------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 8   | 0.512                 | 0.456 | 1.968 | 0.891 | 0.178 | 3.211 | 1.402 | 3.639 | 1.409 | 0.207 |
| 9   | 1.515                 | 0.786 | 3.447 | 2.253 | 1.062 | 6.405 | 4.542 | 5.912 | 3.770 | 0.586 |
| 10  | 0.872                 | 1.082 | 3.081 | 1.620 | -0.004| 5.028 | 2.327 | 6.269 | 2.734 | 0.521 |
| 11  | 1.681                 | 1.380 | 5.042 | 2.480 | 0.677 | 7.971 | 4.292 | 8.665 | 4.142 | 0.901 |

|     | Estimated by Eq(1.2) |       |       |       |       |       |       |       |       |       |
|-----|-----------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1   | 0.822                 | 0.673 | 2.973 | 1.688 | 0.372 | 5.440 | 2.847 | 6.091 | 2.885 | 0.196 |
| 8   | 0.325                 | 0.231 | 1.279 | 0.696 | 0.191 | 2.404 | 1.176 | 2.646 | 1.14  | 0.008 |
| 9   | 1.328                 | 0.56  | 2.758 | 2.058 | 1.076 | 5.597 | 4.316 | 4.919 | 3.501 | 0.387 |
| 10  | 0.685                 | 0.857 | 2.392 | 1.426 | 0.01  | 4.22  | 2.101 | 5.276 | 2.465 | 0.322 |
| 11  | 1.046                 | 0.613 | 2.702 | 1.819 | 0.722 | 5.228 | 3.523 | 5.292 | 3.228 | 0.225 |

AI1 = 5α-androstane-3β-ol
AI2 = 5α-androstane-3α-ol
AI3 = 5α-androstane-17β-ol
AI4 = 5β-androstane-3α,17β-diol
AI5 = 5β-androstane-3β,17β-diol
Two out of seven correlation coefficients were not statistically significant according to the Pearson and Gamma correlation coefficients. The values of the other correlation coefficients were not significantly different by each other (the lowest p-value was obtained when Spearman and Kendall \( \tau_c \) were compared with values of 0.2578 - Eq (1.1), and 0.1862 - Eq (1.2), respectively).

The experimental matrices of the successive chromatographic peaks and those estimated by the models Eq (1.1) and Eq (1.2) are presented in Table 10. The difference between the experimental and estimated values varied from -0.635 (1st peak - experiment no. 9, Eq (1.1)) and 2.172 (2nd peak - experiment no. 10, Eq (1.1)). In most cases (four out of five), the estimated by Eq (1.1) values were greater than the experimental values for experiment no. 10.

**Table 9.** Correlation analysis on resolutions: experimental vs estimated.

| Name             | Correlation coefficient | p-value       | Statistical parameter |
|------------------|-------------------------|---------------|-----------------------|
| Estimated by Eq(1.1), \( n = 40 \) |                          |               |                       |
| Pearson          | \( r = 0.5173 \)       | 6.30\( \cdot \)10^{-4} | \( t_{Prs,1} = 3.72 \) |
| Spearman         | \( \rho = 0.6214 \)   | 1.88\( \cdot \)10^{-5} | \( t_{Spm,1} = 4.89 \) |
| Semi-Q           | \( r_{sQ} = 0.5670 \) | 1.36\( \cdot \)10^{-4} | \( t_{sQ} = 4.24 \) |
| Kendall \( \tau_a \) | \( \tau_{Ken,a} = 0.4462 \) | 5.02\( \cdot \)10^{-5} | \( Z_{Ken,\tau} = 4.05 \) |
| Kendall \( \tau_b \) | \( \tau_{Ken,b} = 0.4462 \) | 5.02\( \cdot \)10^{-5} | \( Z_{Ken,\tau} = 4.05 \) |
| Kendall \( \tau_c \) | \( \tau_{Ken,c} = 0.4350 \) | 7.71\( \cdot \)10^{-5} | \( Z_{Ken,\tau} = 3.95 \) |
| Gamma            | \( \Gamma = 0.4939 \)  | 7.05\( \cdot \)10^{-2} | \( Z_{\Gamma} = 1.81 \) |
| Estimated by Eq(1.2), \( n = 50 \) |                          |               |                       |
| Pearson          | \( r = 0.6185 \)       | 1.70\( \cdot \)10^{-6} | \( t_{Prs,1} = 5.45 \) |
| Spearman         | \( \rho = 0.6786 \)   | 6.12\( \cdot \)10^{-8} | \( t_{Spm,1} = 6.40 \) |
| Semi-Q           | \( r_{sQ} = 0.6478 \) | 3.67\( \cdot \)10^{-7} | \( t_{sQ} = 5.89 \) |
| Kendall \( \tau_a \) | \( \tau_{Ken,a} = 0.4939 \) | 4.18\( \cdot \)10^{-7} | \( Z_{Ken,\tau} = 5.06 \) |
| Kendall \( \tau_b \) | \( \tau_{Ken,b} = 0.4939 \) | 4.18\( \cdot \)10^{-7} | \( Z_{Ken,\tau} = 5.06 \) |
| Kendall \( \tau_c \) | \( \tau_{Ken,c} = 0.4840 \) | 7.07\( \cdot \)10^{-7} | \( Z_{Ken,\tau} = 4.96 \) |
| Gamma            | \( \Gamma = 0.4939 \)  | 1.24\( \cdot \)10^{-2} | \( Z_{\Gamma} = 2.50 \) |

**Table 10.** Resolution matrices of successive chromatographic peaks: experimental vs estimated.

| No. | Experimental peak | Estimated peak by Eq(1.1) | Estimated peak by Eq(1.2) |
|-----|-------------------|---------------------------|---------------------------|
|     | 1st | 2nd | 3rd | 4th | 5th | 1st | 2nd | 3rd | 4th | 5th | 1st | 2nd | 3rd | 4th | 5th |
| 1   | 1.118 | 2.589 | 1.718 | 0.310 | 0.845 | 0.196 | 2.487 | 0.673 | 0.372 | 0.196 |
| 2   | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | | | | |
| 3   | 0.322 | 1.108 | 0.833 | 0.000 | 0.970 | | | | | |
| 4   | 0.528 | 2.217 | 0.427 | 1.442 | 0.228 | | | | | |
| 5   | 0.000 | 1.317 | 0.337 | 0.923 | 0.110 | | | | | |
| 6   | 0.476 | 1.855 | 0.907 | 0.275 | 0.805 | | | | | |
| 7   | 0.178 | 3.254 | 0.585 | 0.000 | 0.977 | | | | | |
| 8   | 0.131 | 2.836 | 2.318 | 0.571 | 0.532 | 0.207 | 1.293 | 0.456 | 0.178 | 0.314 | 0.008 | 1.271 | 0.231 | 0.191 | 0.008 |
| 9   | 2.758 | 4.182 | 1.903 | 1.529 | 0.756 | 0.586 | 2.143 | 0.786 | 1.062 | 0.393 | 0.387 | 2.121 | 0.560 | 1.076 | 0.387 |
| 10  | 0.172 | 2.048 | 0.447 | 0.263 | 0.959 | 0.521 | 2.064 | 1.082 | -0.004 | 1.052 | 0.322 | 2.042 | 0.857 | 0.01 | 0.322 |
| 11  | 2.455 | 4.371 | 1.433 | 0.732 | 0.759 | 0.901 | 2.238 | 1.380 | 0.677 | 0.591 | 0.225 | 2.163 | 0.613 | 0.722 | 0.225 |
The results of the correlation analysis of the resolution matrix of successive chromatographic peaks are presented in Table 11. The values and associated significances of the Pearson and Spearman correlation coefficients sustained the linearity of the relationship between experimental and estimated values. The analysis of the results presented in Table 11 revealed that for both equations the Pearson correlation coefficient is greater than the Spearman correlation coefficient, leading to the conclusion that the investigated response function is a quantitative variable. With a single exception (Gamma correlation analysis), all correlation method sustained the validity of the model from Eq (1.1) and Eq (1.2) \((p \leq 5.54 \times 10^{-3})\). With one exception (Kendall \(\tau_c\), \(\tau_{Ken,c} = 0.4900\)), the values of correlation coefficient were higher than 0.5, indicating moderate to good correlations between experimental and estimated values. Four out of seven correlation coefficients (Kendall \(\tau_a\), Kendall \(\tau_b\), Kendall \(\tau_c\), and Gamma) had values less than or equal with 0.4737, indicating a weak correlation between experimental and estimated by Eq (1.2) values. There could not be identified any statistically significant differences between correlation coefficients presented in Table 11. The lower p-values \((0.2348 - \text{Eq (1.1)}, \text{and } 0.2592 - \text{Eq (1.2)})\) were obtained when Pearson and Kendall \(\tau_c\) correlation coefficients were compared.

### Table 11. Results of correlation analysis: resolution matrix of successive chromatographic peaks (experimental vs estimated).

| Name                  | Correlation coefficient | p-value       | Statistical parameter |
|-----------------------|-------------------------|---------------|-----------------------|
| **Estimated by Eq(1.1), n = 20** |                         |               |                       |
| Pearson               | \(r = 0.7446\)          | \(1.66 \times 10^{-4}\) | \(t_{Prs,1} = 4.73\) |
| Spearman              | \(\rho = 0.6692\)       | \(1.25 \times 10^{-3}\) | \(t_{Spn,1} = 3.82\) |
| Semi-Q                | \(r_{sd} = 0.7059\)     | \(5.06 \times 10^{-4}\) | \(t_Q = 4.23\)       |
| Kendall \(\tau_a\)   | \(\tau_{Ken,a} = 0.5158\) | \(1.47 \times 10^{-3}\) | \(Z_{Ken,\tau_a} = 3.18\) |
| Kendall \(\tau_b\)   | \(\tau_{Ken,b} = 0.5158\) | \(1.47 \times 10^{-3}\) | \(Z_{Ken,\tau_b} = 3.18\) |
| Kendall \(\tau_c\)   | \(\tau_{Ken,c} = 0.4900\) | \(2.52 \times 10^{-3}\) | \(Z_{Ken,\tau_c} = 3.02\) |
| Gamma                 | \(\Gamma = 0.5158\)     | \(1.01 \times 10^{-1}\) | \(Z_{\Gamma} = 1.64\) |
| **Estimated by Eq(1.2), n = 25** |                         |               |                       |
| Pearson               | \(r = 0.6821\)          | \(9.24 \times 10^{-4}\) | \(t_{Prs,1} = 3.96\) |
| Spearman              | \(\rho = 0.6361\)       | \(2.57 \times 10^{-3}\) | \(t_{Spn,1} = 3.50\) |
| Semi-Q                | \(r_{sd} = 0.6587\)     | \(1.59 \times 10^{-3}\) | \(t_Q = 3.71\)       |
| Kendall \(\tau_a\)   | \(\tau_{Ken,a} = 0.4737\) | \(3.50 \times 10^{-3}\) | \(Z_{Ken,\tau_a} = 2.92\) |
| Kendall \(\tau_b\)   | \(\tau_{Ken,b} = 0.4737\) | \(3.50 \times 10^{-3}\) | \(Z_{Ken,\tau_b} = 2.92\) |
| Kendall \(\tau_c\)   | \(\tau_{Ken,c} = 0.4500\) | \(5.54 \times 10^{-3}\) | \(Z_{Ken,\tau_c} = 2.77\) |
| Gamma                 | \(\Gamma = 0.4737\)     | \(1.67 \times 10^{-1}\) | \(Z_{\Gamma} = 1.38\) |

Seven global indicators of separation were introduced and calculated (see Table 2):

- The number distinct compounds on chromatogram DCN - Eq (7);
- The string of standard deviation of retardation factors ordered ascending and estimated by Eq (1) compared with ideal positions of the peaks obtained through experiment RFD - Eq (9);
- The string of sum of the peak resolutions obtained through experiment RSS - Eq (12);
- The squared of effective plate number QN- Eq (13);
- Average peaks separation (into experiment) RSA - Eq (15);
÷ The string of mean resolution calculated with Minkowski experimental peaks RSR - Eq (17);
÷ The string of experimental peaks with minimal resolution QF - Eq (18).

The values associated to the global indicators of separation are presented in Table 12 and 13, respectively. The results of correlation analysis are presented in Table 14 and 15, respectively.

In investigation of the number of distinct compounds on chromatogram, no difference was obtained by both models (Eq (1.1), and Eq (1.2), respectively) for the experiments from 9 to 11 (integer numbers were considered). The same difference of two was obtained between experimental and estimated by both models for experiment no. 8; a difference of 1 for experiment no. 1 when Eq (1.2) was investigated.

Regarding the string of standard deviation of retardation factors ordered ascending and estimated by Eq (1.1) compared with the ideal positions of the peaks obtained through experiment, the lowest difference between experimental and estimated value is obtained by experiment no. 11 (0.001, Eq (1.1)), while the higher difference by the experiment no. 8 (-0.114, Eq (1.1)). The lowest difference of -0.002 was obtained by experiments nos. 8, 9 & 10 and the largest difference of -0.010 was obtained by experiment no. 1 when the Eq (1.2) was investigated.

Table 12. Four (response functions) global indicators on chromatography: experimental vs estimated.

| No. | Experimental DCN | Experimental RFD | Experimental RSS | Experimental QN | Estimated by Eq(1.1), n = 4 DCN | Estimated by Eq(1.1), n = 4 RFD | Estimated by Eq(1.1), n = 4 RSS | Estimated by Eq(1.1), n = 4 QN | Estimated by Eq(1.2), n = 5 DCN | Estimated by Eq(1.2), n = 5 RFD | Estimated by Eq(1.2), n = 5 RSS | Estimated by Eq(1.2), n = 5 QN |
|-----|------------------|------------------|------------------|------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| 1   | 5                | 0.047            | 5.730            | 71.900           | 4 (4.222)                     | 0.057                        | 3.73                          | 83.857                        |                               |                               |                               |                               |
| 2   | 1                | 0.283            | 0.000            | 114.680          |                               |                               |                               |                               |                               |                               |                               |                               |
| 3   | 4                | 0.081            | 2.260            | 108.700          |                               |                               |                               |                               |                               |                               |                               |                               |
| 4   | 5                | 0.055            | 4.610            | 89.550           |                               |                               |                               |                               |                               |                               |                               |                               |
| 5   | 4                | 0.078            | 2.580            | 103.840          |                               |                               |                               |                               |                               |                               |                               |                               |
| 6   | 5                | 0.057            | 3.510            | 83.790           |                               |                               |                               |                               |                               |                               |                               |                               |
| 7   | 3                | 0.097            | 4.020            | 79.280           |                               |                               |                               |                               |                               |                               |                               |                               |
| 8   | 4                | 0.067            | 5.860            | 59.460           | 2 (2.368)                     | 0.181                        | 2.135                         | 98.554                        | 2 (2.200)                     | 0.183                        | 1.703                         | 101.14                        |
| 9   | 5                | 0.036            | 10.370           | 71.240           | 5 (5.168)                     | 0.042                        | 4.574                         | 85.744                        | 5 (5.000)                     | 0.044                        | 4.142                         | 88.326                        |
| 10  | 4                | 0.076            | 2.930            | 80.920           | 4 (4.328)                     | 0.062                        | 3.661                         | 89.591                        | 4 (4.160)                     | 0.064                        | 3.229                         | 92.174                        |
| 11  | 5                | 0.040            | 8.990            | 63.380           | 5 (5.220)                     | 0.039                        | 5.192                         | 79.123                        | 5 (4.650)                     | 0.046                        | 3.725                         | 87.895                        |

DCN = number of distinct compounds on chromatogram;
RFD = string of standard deviation of retardation factors ordered ascending and estimated by Eq(1.1) and Eq(1.2), respectively compared with ideal positions of the peaks obtained through experiment;
RSS = string of sum of the peak resolutions obtained through experiment; QN = squared of effective plate number.

The analysis of the string of sum of the peak resolutions revealed a difference between experimental and estimated of -0.731 (experiment no. 10, Eq (1.1)) and of 5.795 (experiment no. 9, Eq (1.1)). The investigation of Eq (1.2) revealed that the lowest difference of 0.432 was obtained by experiments 8, 9 & 10, and the largest of 2.000 by experiment no. 1. The lowest difference for the squared of effective
plate number response function was obtained in the experiments 8, 9 & 10 (-2.582, Eq (1.2)) and the highest one by the experiment no. 8 (-39.094, Eq (1.2)).

Table 13. Other three global indicators of chromatographic parameters: experimental vs estimated.

| No | Experimental | Estimated by Eq(1.1), n = 4 | Estimated by Eq(1.2), n = 5 |
|----|--------------|----------------------------|----------------------------|
|    | RSA          | RSR           | QF   | RSA          | RSR           | QF   | RSA          | RSR           | QF   |
| 1  | 1.434        | 1.285         | 0.310| 0.932        | 0.636         | 0.075|
| 2  | 0.000        | 0.000         | 0.000| 0.000        | 0.000         | 0.000|
| 3  | 0.566        | 0.401         | 0.000| 0.000        | 0.000         | 0.000|
| 4  | 1.153        | 1.035         | 0.427| 0.000        | 0.000         | 0.000|
| 5  | 0.644        | 0.452         | 0.000| 0.000        | 0.000         | 0.000|
| 6  | 0.878        | 0.778         | 0.275| 0.000        | 0.000         | 0.000|
| 7  | 1.004        | 0.559         | 0.000| 0.000        | 0.000         | 0.000|
| 8  | 1.464        | 1.169         | 0.131| 0.533        | 0.380         | 0.028| 0.425        | 0.240         | 0.000|
| 9  | 2.593        | 2.498         | 1.529| 1.144        | 1.021         | 0.344| 1.035        | 0.881         | 0.293|
| 10 | 0.733        | 0.573         | 0.172| 0.916        | 0.696         | 0.105| 0.807        | 0.556         | 0.054|
| 11 | 2.248        | 2.038         | 0.732| 1.299        | 1.180         | 0.311| 0.931        | 0.704         | 0.138|

RSA = average peaks separation (into experiment); RSR = string of mean resolution calculated with Minkowski of experimental peaks;
QF = string of experimental peaks with minimal resolution.

The last three global response functions had the same difference pattern between experimental and estimated values. The lowest difference is obtained in experiment no. 10 and the highest difference by experiment no. 9 for Eq (1.1) and by experiment no. 8, and experiment no. 11, respectively, for Eq (1.2).

The correlation analysis was applied also on the global quality factors. The lower sample size of experimental data is the major limitation of this analysis and explained the absence of the significance (p \(\geq\) 0.05).

Other three response functions were implemented and computed: resolution divided by the number of effective plates - RSP; informational energy - IEne; and informational entropy - IEnt. The experimental and estimated values are presented in Table 16.

The results of correlation analysis on these response functions are presented in Tables 17 and 18.

The difference of resolution divided by the number of effective plates obtained experimentally and estimated by model from Eq (1.1) varied into a large range: from -3.176 for experiment no. 10 to 36.548 for experiment no. 9 (see Table 15). The variation of the informational energy and informational entropy varied on the same pattern: the lowest difference between experimental and estimated values was obtained by experiment no. 11, while the highest values were obtained by the experiment no. 8 - Eq (1.1).

All response functions presented in Table 16 varied by the same pattern for Eq (1.2): the lowest difference is obtained by experiment no. 8 and the highest one by the experiment no. 1.
Informational response functions, energy and entropy, are parameters that investigated the disorder into the system (in our case the disorders into chromatographic analysis). For both response functions, values lower than 0.41 were obtained, these values being not statistically significant.

**Table 14.** Results of correlation analysis on global quality factors: Eq (1.1), n = 4.

| Name | Correlation coefficient | p-value | Statistical parameter | Name | Correlation coefficient | p-value | Statistical parameter |
|------|-------------------------|---------|-----------------------|------|-------------------------|---------|-----------------------|
| **DCN** | | | | **RSA** | | | |
| Pearson | \( r = 0.8165 \) | 1.80·10^{-1} | \( t_{Prs,1} = 2.0 \) | Pearson | \( r = 0.5905 \) | 4.09·10^{-1} | \( t_{Prs,1} = 1.03 \) |
| Spearman | \( \rho = 0.9428 \) | 5.72·10^{-2} | \( t_{Spn,1} = 4.0 \) | Spearman | \( \rho = 0.6000 \) | 4.00·10^{-1} | \( t_{Spn,1} = 1.06 \) |
| Semi-Q | \( r_Q = 0.8457 \) | 1.23·10^{-1} | \( t_Q = 2.59 \) | Semi-Q | \( r_Q = 0.5952 \) | 4.05·10^{-1} | \( t_Q = 1.05 \) |
| Kendall tau-a | \( \tau_{Ken,a} = 0.6667 \) | 1.75·10^{-1} | \( Z_{Ken,ta} = 1.36 \) | Kendall tau-a | \( \tau_{Ken,a} = 0.3333 \) | 4.97·10^{-1} | \( Z_{Ken,ta} = 0.68 \) |
| Kendall tau-b | \( \tau_{Ken,b} = 0.7303 \) | 1.49·10^{-1} | \( Z_{Ken,tb} = 1.44 \) | Kendall tau-b | \( \tau_{Ken,b} = 0.3333 \) | 4.97·10^{-1} | \( Z_{Ken,tb} = 0.68 \) |
| Kendall tau-c | \( \tau_{Ken,c} = 0.5000 \) | 2.79·10^{-1} | \( Z_{Ken,tc} = 1.08 \) | Kendall tau-c | \( \tau_{Ken,c} = 0.2500 \) | 6.10·10^{-1} | \( Z_{Ken,tc} = 0.51 \) |
| Gamma | \( \Gamma = 1.0000 \) | 4.15·10^{-2} | \( Z_{\Gamma} = 2.04 \) | Gamma | \( \Gamma = 0.3333 \) | 8.21·10^{-1} | \( Z_{\Gamma} = 0.23 \) |
| **RFD** | | | | **RSR** | | | |
| Pearson | \( r = 0.5434 \) | 4.56·10^{-1} | \( t_{Prs,1} = 0.92 \) | Pearson | \( r = 0.7118 \) | 2.88·10^{-1} | \( t_{Prs,1} = 2.05 \) |
| Spearman | \( \rho = 0.6000 \) | 4.00·10^{-1} | \( t_{Spn,1} = 1.06 \) | Spearman | \( \rho = 0.6000 \) | 3.46·10^{-1} | \( t_{Spn,1} = 1.06 \) |
| Semi-Q | \( r_Q = 0.5710 \) | 4.29·10^{-1} | \( t_Q = 0.98 \) | Semi-Q | \( r_Q = 0.6535 \) | 5.73·10^{-1} | \( t_Q = 1.22 \) |
| Kendall tau-a | \( \tau_{Ken,a} = 0.3333 \) | 4.97·10^{-1} | \( Z_{Ken,ta} = 0.68 \) | Kendall tau-a | \( \tau_{Ken,a} = 0.3333 \) | 4.47·10^{-1} | \( Z_{Ken,ta} = 0.68 \) |
| Kendall tau-b | \( \tau_{Ken,b} = 0.3333 \) | 4.97·10^{-1} | \( Z_{Ken,tb} = 0.68 \) | Kendall tau-b | \( \tau_{Ken,b} = 0.3333 \) | 4.97·10^{-1} | \( Z_{Ken,tb} = 0.68 \) |
| Kendall tau-c | \( \tau_{Ken,c} = 0.2500 \) | 6.10·10^{-1} | \( Z_{Ken,tc} = 0.51 \) | Kendall tau-c | \( \tau_{Ken,c} = 0.2500 \) | 6.10·10^{-1} | \( Z_{Ken,tc} = 0.51 \) |
| Gamma | \( \Gamma = 0.3333 \) | 8.21·10^{-1} | \( Z_{\Gamma} = 0.23 \) | Gamma | \( \Gamma = 0.3333 \) | 8.21·10^{-1} | \( Z_{\Gamma} = 0.23 \) |
| **RSS** | | | | **QF** | | | |
| Pearson | \( r = 0.5906 \) | 4.09·10^{-1} | \( t_{Prs,1} = 1.04 \) | Pearson | \( r = 0.8936 \) | 1.06·10^{-1} | \( t_{Prs,1} = 2.82 \) |
| Spearman | \( \rho = 0.6000 \) | 4.00·10^{-1} | \( t_{Spn,1} = 1.13 \) | Spearman | \( \rho = 1.0000 \) | 5.47·10^{-2} | \( t_{Spn,1} = 4.10 \) |
| Semi-Q | \( r_Q = 0.5953 \) | 4.05·10^{-1} | \( t_Q = 1.05 \) | Semi-Q | \( r_Q = 0.9453 \) | 6.68·10^{-1} | \( t_Q = 2.82 \) |
| Kendall | \( \tau_{Ken,a} = 0.3333 \) | 1.97·10^{-1} | \( Z_{Ken,ta} = 0.68 \) | Kendall | \( \tau_{Ken,a} = 1.0000 \) | 4.15·10^{-2} | \( Z_{Ken,ta} = 2.04 \) |
| Kendall | \( \tau_{Ken,b} = 0.3333 \) | 4.97·10^{-1} | \( Z_{Ken,tb} = 0.68 \) | Kendall | \( \tau_{Ken,b} = 1.0000 \) | 4.15·10^{-2} | \( Z_{Ken,tb} = 2.04 \) |
| Kendall | \( \tau_{Ken,c} = 0.2500 \) | 6.10·10^{-1} | \( Z_{Ken,tc} = 0.51 \) | Kendall | \( \tau_{Ken,c} = 0.7500 \) | 1.26·10^{-1} | \( Z_{Ken,tc} = 1.53 \) |
| Gamma | \( \Gamma = 0.3333 \) | 8.21·10^{-1} | \( Z_{\Gamma} = 0.23 \) | Gamma | \( \Gamma = 1.0000 \) | 4.15·10^{-2} | \( Z_{\Gamma} = 2.04 \) |
| **QN** | | | | | | | |
| Pearson | \( r = -0.1588 \) | 9.85·10^{-1} | \( t_{Prs,1} = 0.22 \) | | n = sample size; |
| Spearman | \( \rho = -0.2000 \) | 8.22·10^{-1} | \( t_{Spn,1} = 0.29 \) | DCN = number of distinct compounds on chromatogram; |
| Semi-Q | \( r_Q = 0.1782 \) | 8.00·10^{-1} | \( t_Q = 0.25 \) | RFD = string of standard deviation of retardation factors |
| Kendall tau-a | \( \tau_{Ken,a} = 0.0000 \) | 1.00 | \( Z_{Ken,ta} = 0.00 \) | estimated by Eq(1) ordered ascending compared with ideal |
| Kendall tau-b | \( \tau_{Ken,b} = 0.0000 \) | 1.00 | \( Z_{Ken,tb} = 0.00 \) | positions of the peaks obtained through experiment; |
| Kendall tau-c | \( \tau_{Ken,c} = 0.0000 \) | 1.00 | \( Z_{Ken,tc} = 0.00 \) | RSS = string of sum of the peak resolutions obtained |
| Gamma | \( \Gamma = 0.0000 \) | 1.00 | \( Z_{\Gamma} = 0.00 \) | through experiment; |
| | | | | QN = squared of effective plate number; |
| | | | | RSR = string of Minkowski mean resolution of experimental |
| | | | | peaks; |
| | | | | QF = string of experimental peaks with minimal resolution. |
Table 15. Results of correlation analysis on global quality factors: Eq (1.2), n = 5.

| Name     | Correlation coefficient | p-value | Statistical parameter | Name     | Correlation coefficient | p-value | Statistical parameter |
|----------|-------------------------|---------|-----------------------|----------|-------------------------|---------|-----------------------|
| DCN      | r = 0.7454              | 1.48·10^-1 | t_{Prs,1} = 3.75     | RSA      | r = 0.4698              | 4.25·10^-1 | t_{Prs,1} = 0.92     |
|          | r = 0.4722              | 4.22·10^-1 | t_{Spm,1} = 0.93     |          | r = 0.5000              | 3.91·10^-1 | t_{Spm,1} = 1.00     |
|          | r_dQ = 0.5933           | 2.92·10^-1 | t_{Q} = 1.28         |          | r_dQ = 0.4874           | 4.08·10^-1 | t_{Q} = 0.96         |
| Kendall  | τ_{Ken,a} = 0.3000     | 4.62·10^-1 | Z_{Ken,ta} = 0.73    | Kendall  | τ_{Ken,a} = 0.4000     | 3.27·10^-1 | Z_{Ken,ta} = 0.98    |
| Kendall  | τ_{Ken,b} = 0.3162     | 4.49·10^-1 | Z_{Ken,rb} = 0.76    | Kendall  | τ_{Ken,b} = 0.4000     | 3.27·10^-1 | Z_{Ken,rb} = 0.98    |
| Kendall  | τ_{Ken,c} = 0.2400     | 5.44·10^-1 | Z_{Ken,rc} = 0.61    | Kendall  | τ_{Ken,c} = 0.3200     | 4.33·10^-1 | Z_{Ken,rc} = 0.78    |
| Gamma    | Γ = 0.4286             | 6.53·10^-1 | Z_Γ = 0.45           | Gamma    | Γ = 0.4000              | 6.95·10^-1 | Z_Γ = 0.39           |
| RFD      | r = 0.5520             | 3.35·10^-1 | t_{Prs,1} = 1.15     | RSR      | r = 0.6827              | 2.04·10^-1 | t_{Prs,1} = 2.62     |
|          | r = 0.9000             | 3.74·10^-2 | t_{Spm,1} = 3.58     |          | r = 0.9000              | 3.74·10^-2 | t_{Spm,1} = 3.58     |
|          | r_dQ = 0.7049           | 1.84·10^-1 | t_{Q} = 1.72         |          | r_dQ = 0.7838           | 1.17·10^-1 | t_{Q} = 2.19         |
| Kendall  | τ_{Ken,a} = 0.8000     | 5.00·10^-2 | Z_{Ken,ta} = 1.96    | Kendall  | τ_{Ken,a} = 0.8000     | 5.00·10^-2 | Z_{Ken,ta} = 1.96    |
| Kendall  | τ_{Ken,b} = 0.8000     | 5.00·10^-2 | Z_{Ken,rb} = 1.96    | Kendall  | τ_{Ken,b} = 0.8000     | 5.00·10^-2 | Z_{Ken,rb} = 1.96    |
| Kendall  | τ_{Ken,c} = 0.6400     | 1.17·10^-1 | Z_{Ken,rc} = 1.57    | Kendall  | τ_{Ken,c} = 0.6400     | 1.17·10^-1 | Z_{Ken,rc} = 1.57    |
| Gamma    | Γ = 0.8000             | 1.17·10^-1 | Z_Γ = 1.57           | Gamma    | Γ = 0.8000              | 1.17·10^-1 | Z_Γ = 1.57           |
| RSS      | r = 0.4691             | 4.25·10^-1 | t_{Prs,1} = 0.92     | QF       | r = 0.9871              | 1.76·10^-3 | t_{Prs,1} = 10.67    |
|          | r = 0.5000             | 3.91·10^-1 | t_{Spm,1} = 1.00     |          | r = 1.0000              | 1.24·10^-2 | t_{Spm,1} = 5.41     |
|          | r_dQ = 0.4843           | 4.08·10^-1 | t_{Q} = 0.96         |          | r_dQ = 0.9935           | 6.26·10^-4 | t_{Q} = 15.14        |
| Kendall  | τ_{Ken,a} = 0.4000     | 3.27·10^-1 | Z_{Ken,ta} = 0.98    | Kendall  | τ_{Ken,a} = 1.0000     | 1.43·10^-2 | Z_{Ken,ta} = 2.45    |
| Kendall  | τ_{Ken,b} = 0.4000     | 3.27·10^-1 | Z_{Ken,rb} = 0.98    | Kendall  | τ_{Ken,b} = 1.0000     | 1.43·10^-2 | Z_{Ken,rb} = 2.45    |
| Kendall  | τ_{Ken,c} = 0.3200     | 4.33·10^-1 | Z_{Ken,rc} = 0.78    | Kendall  | τ_{Ken,c} = 0.8000     | 5.00·10^-2 | Z_{Ken,rc} = 1.96    |
| Gamma    | Γ = 0.4000             | 6.95·10^-1 | Z_Γ = 0.39           | Gamma    | Γ = 1.0000              | 1.43·10^-2 | Z_Γ = 2.45           |
| QN       | r = -0.4189           | 4.82·10^-1 | t_{Prs,1} = 0.80     |          | n = sample size; DCN = number of distinct compounds on chromatogram; |
|          | r = -0.3000            | 6.24·10^-1 | t_{Spm,1} = 0.54     |          | RFD = string of standard deviation of retardation factors ordered ascending and estimated by Eq(1) compared with ideal positions of the peaks obtained through experiment; |
|          | r_dQ = 0.3545          | 5.58·10^-1 | t_{Q} = 0.66         | Kendall  | τ_{Ken,a} = 0.2000     | 6.24·10^-1 | Z_{Ken,ta} = 0.49    |
| Kendall  | τ_{Ken,b} = 0.2000     | 6.24·10^-1 | Z_{Ken,rb} = 0.49    | Kendall  | τ_{Ken,b} = 0.2000     | 6.24·10^-1 | Z_{Ken,rb} = 0.49    |
| Kendall  | τ_{Ken,c} = 0.1600     | 7.05·10^-1 | Z_{Ken,rc} = 0.39    | Kendall  | τ_{Ken,c} = 0.1600     | 7.05·10^-1 | Z_{Ken,rc} = 0.39    |
| Gamma    | Γ = 0.2000             | 9.22·10^-1 | Z_Γ = 0.10           | Kendall  | τ_{Ken,c} = 0.1600     | 7.05·10^-1 | Z_{Ken,rc} = 0.39    |
Table 16. Resolution ratio, informational energy and entropy: experimental vs estimated.

| No. | Experimental RSP | IEnt | IEne | Estimated by Eq(1.1), n = 4 RSP | IEnt | IEne | Estimated by Eq(1.2), n = 5 RSP | IEnt | IEne |
|-----|------------------|------|------|---------------------------------|------|------|---------------------------------|------|------|
| 1   | 31.900           | 4.000| 16.000| 17.678                          | 10.407| 2.67 |
| 2   | 0.000            | 11.610| 0.000|                                |      |      |
| 3   | 8.300            | 11.610| 0.000|                                |      |      |
| 4   | 20.600           | 8.000 | 8.000|                                |      |      |
| 5   | 9.900            | 11.610| 0.000|                                |      |      |
| 6   | 16.800           | 8.000 | 8.000|                                |      |      |
| 7   | 20.300           | 11.610| 0.000|                                |      |      |
| 8   | 39.400           | 4.000 | 16.000| 11.096                          | 2.560| 8.024| 11.754                          | 0.00 |
| 9   | 58.200           | 2.000 | 18.000| 21.652                          | 7.338| 9.280| 18.582                          | 6.40 |
| 10  | 14.500           | 11.610| 0.000| 17.676                          | 9.360| 4.800| 14.604                          | 1.92 |
| 11  | 56.700           | 4.750 | 14.000| 27.285                          | 5.203| 13.565| 16.852                          | 9.902| 3.78 |

RSP = resolution divided by the number of effective plates;
IEnt = informational energy;
IEne = informational entropy.

Table 17. Results of correlation analysis for response functions presented in Table 15: Eq (1.1), n = 4.

| Name | Correlation coefficient | p-value | Statistical parameter | Name | Correlation coefficient | p-value | Statistical parameter |
|------|-------------------------|---------|-----------------------|------|-------------------------|---------|-----------------------|
| RSP  | r = 0.5326              | 4.67·10⁻¹ | tPrs₁ = 0.90         | IEnt| r = 0.3188              | 6.81·10⁻¹ | tPrs₁ = 0.48         |
|      | Spearman ρ = 0.6000     | 4.00·10⁻¹ | tSpm₁ = 1.06         |      | Spearman ρ = 0.0000     | 1.00     | tSpm₁ = 0.00         |
|      | Semi-Q τq = 0.5653      | 4.35·10⁻¹ | tq = 0.97             |      | Semi-Q τq = 0.0000      | 1.00     | tq = 0.00             |
|      | Kendall tau-a τKen,a = 0.3333 | 4.97·10⁻¹ | ZKen,ta = 0.68      | Kendall tau-a τKen,a = 0.0000 | 1.00 | ZKen,ta = 0.00 |
|      | Kendall tau-b τKen,b = 0.3333 | 4.97·10⁻¹ | ZKen,tb = 0.68      | Kendall tau-b τKen,b = 0.0000 | 1.00 | ZKen,tb = 0.00 |
|      | Kendall tau-c τKen,c = 0.2500 | 6.10·10⁻¹ | ZKen,tc = 0.51      | Kendall tau-c τKen,c = 0.0000 | 1.00 | ZKen,tc = 0.00 |
|      | Gamma Γ = 0.3333        | 8.21·10⁻¹ | ZΓ = 0.23            | Gamma Γ = 0.0000      | 1.00 | ZΓ = 0.00             |

IEnt
| Pearson r = 0.2962 | 7.04·10⁻¹ | tPrs₁ = 0.44 | RSP = resolution divided by the number of effective plates;
| Spearman ρ = 0.0000 | 1.00 | tSpm₁ = 0.00 | IEnt = informational energy;
| Semi-Q τq = 0.0000 | 1.00 | tq = 0.00 | IEne = informational entropy;
| Kendall τKen,a = 0.0000 | 1.00 | ZKen,ta = 0.00 | n = sample size.
| Kendall τKen,b = 0.0000 | 1.00 | ZKen,tb = 0.00 |
| Kendall τKen,c = 0.0000 | 1.00 | ZKen,tc = 0.00 |
| Gamma Γ = 0.0000 | 1.00 | ZΓ = 0.00 |
Table 18. Results of correlation analysis for response functions presented in Table 15: Eq (1.2), n = 5.

| Name  | Correlation coefficient | p-value | Statistical parameter | Name  | Correlation coefficient | p-value | Statistical parameter |
|-------|-------------------------|---------|------------------------|-------|-------------------------|---------|------------------------|
| RSP   | Pearson: \( r = 0.2864 \) | 6.40·10^{-1} | \( t_{Prs,1} = 0.52 \) | IEnt  | Pearson: \( r = 0.3770 \) | 5.32·10^{-1} | \( t_{Prs,1} = 0.71 \) |
|       | Spearman: \( \rho = 0.5000 \) | 3.91·10^{-1} | \( t_{Spm,1} = 1.00 \) |       | Spearman: \( \rho = 0.4104 \) | 4.92·10^{-1} | \( t_{Spm,1} = 0.78 \) |
|       | Semi-Q: \( r_{pq} = 0.3784 \) | 5.30·10^{-1} | \( t_{pq} = 0.71 \) |       | Semi-Q: \( r_{pq} = 0.3934 \) | 5.12·10^{-1} | \( t_{pq} = 0.74 \) |
|       | Kendall tau-a: \( \tau_{Ken,a} = 0.4000 \) | 3.27·10^{-1} | \( Z_{Ken,ta} = 0.98 \) |       | Kendall tau-a: \( \tau_{Ken,a} = 0.3000 \) | 4.62·10^{-1} | \( Z_{Ken,ta} = 0.73 \) |
|       | Kendall tau-b: \( \tau_{Ken,b} = 0.4000 \) | 3.27·10^{-1} | \( Z_{Ken,lb} = 0.98 \) |       | Kendall tau-b: \( \tau_{Ken,b} = 0.3162 \) | 4.48·10^{-1} | \( Z_{Ken,lb} = 0.76 \) |
|       | Kendall tau-c: \( \tau_{Ken,c} = 0.3200 \) | 4.33·10^{-1} | \( Z_{Ken,tc} = 0.78 \) |       | Kendall tau-c: \( \tau_{Ken,c} = 0.2400 \) | 5.44·10^{-1} | \( Z_{Ken,tc} = 0.61 \) |
| Gamma | \( \Gamma = 0.4000 \) | 6.95·10^{-1} | \( Z_{\Gamma} = 0.39 \) | Gamma | \( \Gamma = 0.3333 \) | 7.85·10^{-1} | \( Z_{\Gamma} = 0.27 \) |

The summary of the acceptance of the linear relationships between experimental and estimated values by the Eq (1.1) and Eq (1.2) for the investigated response functions is presented in Table 19.

Experiments Quality Assessment

RF, RFO, RSM, RSO, and QF chromatographic response functions can be accepted as being dependent on mobile phase composition with a good confidence, according to the results presented in Table 19. This partial conclusion can be used now backward, in order to see what is wrong (if there is something) in the experiments. Table 20 presented the biggest 20% differences between experimental and predicted by the models values. The Diff column from Table 20 contains relative differences calculated as follows:

\[
\text{Diff} = 50 \cdot |\text{Exp-Est}|/(\text{Exp+Est}) \%
\]  \quad (18)

Results from Table 20 can be analyzed in terms of relative deviation using Eq (18) if the values of relative deviation obtained for a given model and chromatographic response functions are ordered by rank.
Table 19. Summary of validation the response functions estimated by the model from Eq (1.1) and Eq (1.2), respectively.

| Parameter | Pearson | Spearman | Semi-Q | Kendall τa | Kendall τb | Kendall τc | Gamma |
|-----------|---------|----------|--------|------------|------------|------------|-------|
| RF        | ✓       | ✓        | ✓      | ✓          | ✓          | ✓          | ✓     |
| RFO       | ✓       | ✓        | ✓      | ✓          | ✓          | ✓          | ✓     |
| RSM       | ✓       | ✓        | ✓      | ✓          | ✓          | ✓          | ✓     |
| RSO       | ✓       | ✓        | ✓      | ✓          | ✓          | ✓          | x     |
| QF        | x       | x        | x      | ✓          | ✓          | ✓          | x     |
| DCN       | x       | x        | x      | x          | x          | x          | ✓     |
| RFD       | x       | x        | x      | x          | x          | x          | x     |
| RSS       | x       | x        | x      | x          | x          | x          | x     |
| QN        | x       | x        | x      | x          | x          | x          | x     |
| RSA       | x       | x        | x      | x          | x          | x          | x     |
| RSR       | x       | x        | x      | x          | x          | x          | x     |
| RSP       | x       | x        | x      | x          | x          | x          | x     |
| IEne      | x       | x        | x      | x          | x          | x          | x     |
| IEnt      | x       | x        | x      | x          | x          | x          | x     |

✓ = statistical significant at a significance level of 5%; x = statistical insignificant at a significance level of 5%;
RF = retardation factor; RFO = retardation factor ordered ascending by the chromatographic peak;
RSM = resolution of pairs of compounds;
RSO = resolution matrix of successive chromatographic peaks;
DCN = number of distinct compounds on chromatogram;
RFD = string of standard deviation of retardation factors ordered ascending and estimated by Eq(1.1), Eq(1.2) respectively, compared with ideal positions of the peaks obtained through experiment;
RSS = string of sum of the peak resolutions obtained through experiment;
QN = squared of effective plate number;
RSA = average peaks separation (into experiment);
RSR = string of mean resolution calculated with Minkowski experimental peaks;
QF = string of experimental peaks with minimal resolution;
RSP = resolution divided by the number of effective plates;
IEnt = informational energy; IEne = informational entropy;
Table 20. Summary of validation the response functions estimated by the model from Eq (1.1) and Eq (1.2), respectively.

| No | Model | CRF | Estimated | Experimental | Difference (%) | Group Rank | Exp No |
|----|-------|-----|-----------|--------------|----------------|------------|-------|
| 1  | Eq (1.1) | RF  | 0.505     | 0.181        | 23.62          | 1          | 11    |
| 2  | Eq (1.1) | RF  | 0.253     | 0.709        | 23.70          | 2          | 11    |
| 3  | Eq (1.1) | RF  | 0.216     | 0.072        | 25.00          | 3          | 8     |
| 4  | Eq (1.1) | RF  | 0.203     | 0.060        | 27.19          | 4          | 8     |
| 5  | Eq (1.1) | RSM | 0.456     | 2.318        | 33.56          | 1          | 8     |
| 6  | Eq (1.1) | RSM | 1.38      | 7.191        | 33.90          | 2          | 11    |
| 7  | Eq (1.1) | RSM | 8.665     | 1.433        | 35.81          | 3          | 11    |
| 8  | Eq (1.1) | RSM | 0.677     | 4.371        | 36.59          | 4          | 11    |
| 9  | Eq (1.1) | RSM | -0.004    | 0.263        | 51.54          | 5          | 10    |
| 10 | Eq (1.1) | RFO | 0.393     | 0.756        | 15.80          | 1          | 9     |
| 11 | Eq (1.1) | RFO | 0.347     | 0.683        | 16.31          | 2          | 9     |
| 12 | Eq (1.1) | RFO | 0.219     | 0.072        | 25.26          | 3          | 8     |
| 13 | Eq (1.1) | RFO | 0.200     | 0.06         | 26.92          | 4          | 8     |
| 14 | Eq (1.1) | RSM | 0.586     | 2.758        | 32.48          | 1          | 9     |
| 15 | Eq (1.1) | RSM | 0.456     | 2.318        | 33.56          | 2          | 8     |
| 16 | Eq (1.1) | RSM | -0.004    | 0.263        | 51.54          | 3          | 10    |
| 17 | Eq (1.1) | QF  | 0.028     | 0.131        | 32.39          | 1          | 8     |
| 18 | Eq (1.2) | RF  | 0.311     | 0.709        | 19.51          | 1          | 11    |
| 19 | Eq (1.2) | RF  | 0.322     | 0.759        | 20.21          | 2          | 11    |
| 20 | Eq (1.2) | RF  | 0.438     | 0.181        | 20.76          | 3          | 11    |
| 21 | Eq (1.2) | RF  | 0.215     | 0.072        | 24.91          | 4          | 8     |
| 22 | Eq (1.2) | RF  | 0.220     | 0.060        | 28.57          | 5          | 8     |
| 23 | Eq (1.2) | RSM | 2.646     | 14.473       | 34.54          | 1          | 8     |
| 24 | Eq (1.2) | RSM | 1.819     | 10.234       | 34.91          | 2          | 11    |
| 25 | Eq (1.2) | RSM | 0.196     | 1.118        | 35.08          | 3          | 1     |
| 26 | Eq (1.2) | RSM | 0.722     | 4.371        | 35.82          | 4          | 11    |
| 27 | Eq (1.2) | RSM | 0.387     | 2.758        | 37.69          | 5          | 9     |
| 28 | Eq (1.2) | RSM | 0.325     | 2.392        | 38.04          | 6          | 8     |
| 29 | Eq (1.2) | RSM | 0.231     | 2.318        | 40.94          | 7          | 8     |
| 30 | Eq (1.2) | RSM | 0.613     | 7.191        | 42.15          | 8          | 11    |
| 31 | Eq (1.2) | RSM | 0.008     | 0.131        | 44.24          | 9          | 8     |
| 32 | Eq (1.2) | RSM | 0.010     | 0.263        | 46.34          | 10         | 10    |
| 33 | Eq (1.2) | RSM | 0.289     | 0.573        | 16.47          | 1          | 9     |
| 34 | Eq (1.2) | RFO | 0.364     | 0.756        | 17.50          | 2          | 9     |
| 35 | Eq (1.2) | RFO | 0.317     | 0.683        | 18.30          | 3          | 9     |
| 36 | Eq (1.2) | RFO | 0.219     | 0.072        | 25.26          | 4          | 8     |
| 37 | Eq (1.2) | RFO | 0.215     | 0.060        | 28.18          | 5          | 8     |
| 38 | Eq (1.2) | RSM | 0.231     | 2.318        | 40.94          | 1          | 8     |
A qualitative measure of experiments results can be obtained by constructing the graphical representation of experiments based on the rank sums of relative deviations. Figure 1 presents the plot of rank sums deviations for the experiments included in the estimation.

**Figure 1.** Rank sums for relative deviations of investigated experiments.

The analysis of values presented in Figure 1 showed that over 60 relative deviation ranks sum are recorded for experiment no. 8 (about 46.4%), followed by over 30 relative deviation ranks sum for experiment no. 11 (about 24.6%, about half relative to the experiment no. 8). Considering a normal distribution of unbiased error, the expected distribution of deviations rank sum is uniform. Thus, the expected frequencies for ranks sums in investigated experiments are no more than 25% (no more than 34 relative deviation ranks sum). Concluding, the results obtained for deviations of rank sums suggest that results from experiment no. 8 are questionable.

**Conclusions**

The analyses of the data presented in Tables 19, 20 and Figure 1 leads to the following conclusions:

1. The model presented in Eq (1.2) seems to be more reliable for the estimation of chromatographic response functions on investigated androstane isomers. Four response
functions (RF - retardation factor; RFO - retardation factor ordered ascending by the chromatographic peak; RSM - resolution of pairs of compounds; QF - string of experimental peaks with minimal resolution) revealed statistically significant linear relationships between experimental and estimated values.

2. The models presented in Eq (1.1) is valid and reliable in investigation of retardation factor, retardation factor ordered ascending by the chromatographic response, resolution of pairs of compounds and resolution matrix of successive chromatographic peaks;

3. Good performances are obtained in estimation of resolution of pairs of compounds but the relationship between experimental and estimated values by Eq (1.1) and Eq (1.2) could be questionable due to the absence of significantly statistic Gamma correlation coefficient;

4. Some estimation abilities were observed in investigation of the string of standard deviation of retardation factors ordered ascending estimated by Eq (1.2) compared with ideal positions of the peaks obtained through experiment; and of the string of Minkowski type mean resolution calculated by Eq (1.2) with experimental peaks. These two chromatographic response functions seem to be qualitative and rank variables.

5. Two global response functions for the separation, abbreviated as QF and DCN recorded a weak acceptance in investigation of Eq (1.1). Thus, QF are rejected at 95% confidence by Spearman (with 5.47% error), Semi-Q (6.68% error), Pearson (with 10.6% error) and Kendall τc (with 12.6% error) even if the correlations are good (over 0.75). The small dimension of the sample size, not grater enough to provide statistical significance of the obtained correlations, explained with a good confidence the rejection of these correlations. Note that QF chromatographic response function is in fact a minimum function of resolutions of the separation, resolutions that are accepted by the model from Eq (1.1) - see 2nd conclusion. DCN is statistically significant by the Goodman-Kruskal method (concordant vs. discordant) and is near to be statistically significant by the Spearman method (5.72% error). Thus, the rejection is recorded for a quantitative correlation, but a possible acceptance is seen by the qualitative correlation. Again, small sample size is against of a solid statistical conclusion for DCN.

6. The results presented in Experiments Quality Assessment subsection sustain the hypothesis that the proposed equations (Eq (1.1) and Eq (1.2), respectively) could be used in order to verify the quality of experimental data. The results obtained for deviations of rank sums suggest that the experimental data of the experiment no. 8 are questionable.

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References and Notes

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