*Polish J. of Environ. Stud. Vol. 19, No. 5 (2010), 1071-1075*

*Short Communication*

# Chemometric Processing of Ion Chromatograms – Application to Comparative Analysis of Polish Bottled Mineral and Spring Waters

**Eliza Blicharska[1]*, Łukasz Komsta[2], Ryszard Kocjan[1],
Anna Gumieniczek[2], Agnieszka Wiśniewska[1]**

[1]Department of Analytical Chemistry, Medical University of Lublin, Staszica 6, 20-081 Lublin, Poland
[2]Department of Medicinal Chemistry, Medical University of Lublin, Jaczewskiego 4, 20-090 Lublin, Poland

*Received: 19 December 2009*
*Accepted: 22 April 2010*

## Abstract

The fingerprinting concept that treats a chromatogram as a unique signal without peak identifying has been used in chromatographic comparative analysis. So far, no such approaches of ion chromatography have been published. Therefore, the aim of our work was to perform ion chromatographic analysis of five metals: Fe(III), Cu(II), Ni(II), Zn(II), and Mn(II) in 30 bottled mineral and spring waters available in the Polish market. The fingerprinting approach performed well and its main advantage is no need to perform the calibration procedure of each chromatographed ion. By investiging loadings, intercorrelated and orthogonal peaks were identified. We discuss the chemometric steps needed before comparison: smoothing, baseline removal, and warping.

**Keywords:** ion chromatography, water analysis, drinking water, chemometrics, metals

## Introduction

Research on heavy metal concentrations in environmental samples is now a widely elaborated topic [1]. The attention of researchers is often focused on drinking water, both in the case of microelements and toxic metals [2-5]. In the literature one can find drinking water investigations from all over the world. The examples of such studies in Poland are: nitrates and nitrites [6, 7], selenium [8], zinc and copper [9], manganese and iron [10], several metals with cluster analysis [11], and chromium [12, 13]. Besides flame atomic absorption spectrometry (FAAS), ion chromatography (IC) is often used to investigate metal content in different samples.

The development of computer technology in the 1990s resulted in an ability to collect large amounts of analytical data and process it very quickly. This connects with popular interests in chemometrics, also in the case of chromatography [14]. Although the majority of the investigations rely on identifying the peaks and quantitative evaluation of them, the comparative analysis of the samples can be done by so-called fingerprinting, where chromatogram is treated as a unique signal-like vector [15]. The successful and proper processing of such signals rely on correct preprocessing. Chromatograms must be denoised [16, 17], warped [18, 19], and filtered from baseline drifts [15]. The main advantage of such processing is no need to identify and quantify peaks and – in some limits – compare chromatograms with a different number of peaks. Additionally, the multivariate analysis of classical quantitative data matrix leads to problems when some concentrations are positive, but are below the detection limit. This problem does not exist in the case of fingerprinting.

To our knowledge, the fingerprinting approach has not been used in ion chromatography analysis. Therefore, the aim of our study was to investigate the usefullness of such an approach in the case of Polish bottled (spring and mineral) waters.

---

*e-mail: bayrena@tlen.pl

## Experimental

The following spring and mineral waters (purchased in a local food store in Lublin, Poland) were analyzed (reference letters or digits in parentheses): Aleksandria Aro (A), Alvin (B), Aqua Minerale (C), Aquarel (D), Arctic (E), Babia Góra (F), Cisowianka (G), Czantoria (H), Dobrawa (I), Eco (J), Górska Natura (K), Kazimierska (L), Kinga Pienińska (M), Kropla Beskidu (N), Królewska (O), Krynica Zdrój (P), Muszyna (Q), Muszynianka (R), Nałęczowianka (S), Piwniczanka (T), Polaris (U), Primavera (V), Rogowiec (W), Świętokrzyska (X), Świtezianka (Y), Ustronianka (Z), Veroni Mineral (1), Vita (2), Źródło Tymbark (3), and Żywiec Zdrój (4).

The waters were directly (without dilution) analyzed using a Dionex DC-500 IC ion chromatograph with online post-column derivatization and spectrophotometric detection. The analysis was performed using a dedicated pre-column (CGP 5A) and column (CS 5A). The aqueous mobile phase contained 35 mol/l pyridinecarboxylic acid (PDCA), 0.37 mol/l formic acid, 0.33 mol/l KOH, and 28 mmol/l $K_2SO_4$.

The derivatization was carried out using an aqueous solution containing 2.8 mmol/l 4-(2-pyrridazo)resorcinol (PAR), 0.3 mol/l $CaCO_3$, 1 mol/l dimethylaminethanol and 0.5 mol/l of ammonia.

Each sample was injected 5 times, which resulted in 150 chromatograms. Each chromatogram contained peaks corresponding to the following ions: Fe(III), Cu(II), Ni(II), Zn(II), and Mn(II). The mean content of determined metals is presented in Table 1.

## Data Processing

The chromatograms were arranged in a matrix of 150 rows x 5,100 columns (each chromatogram contained 5,100 data points). The chemometric processing was done in GNU R 2.82 computational environment (www.r-project.org). In the beginning, the first 750 columns of the matrix were cut out from the dataset. Next, the Savitzky-Golay smoother was applied [17], then baseline was filtered with asymmetric penalized least squares [15]. There was no need to perform warping of the peaks. Processed chromatograms were analyzed by means of classical unscaled principal component analysis by "prcomp" R function.

Table 1. The determined Fe, Cu, Ni, Zn, and Mn content (μg/kg) in analyzed mineral and spring water. For sample numbering (A – 5) see Experimental section.

| | Fe | Cu | Ni | Zn | Mn |
|---|---|---|---|---|---|
| A | 4.6 | 0.1 | 0.3 | 1.42 | 0.2 |
| B | 2.46 | 0.1 | 0.3 | 333.4 | 0.2 |
| C | 6.46 | 0.1 | 0.3 | 2.54 | 0.2 |
| D | 6.32 | 0.1 | 0.3 | 2.46 | 0.2 |
| E | 6.36 | 0.1 | 0.3 | 13.54 | 5.32 |
| F | 1.52 | 1.62 | 0.3 | 21.46 | 2.52 |
| G | 8.58 | 0.1 | 0.3 | 1.96 | 0.2 |
| H | 3.64 | 13.32 | 2 | 22.44 | 0.2 |
| I | 5.98 | 0.1 | 0.3 | 90.6 | 1.02 |
| J | 1.64 | 1.12 | 0.3 | 32.48 | 0.2 |
| K | 2.66 | 0.1 | 0.3 | 4.5 | 0.2 |
| L | 5.04 | 0.1 | 0.3 | 2.22 | 0.2 |
| M | 8.92 | 0.1 | 0.3 | 2.2 | 0.2 |
| N | 3.58 | 0.1 | 0.3 | 1.76 | 0.2 |
| O | 5.02 | 0.1 | 5.7 | 13.36 | 0.2 |
| P | 2.76 | 1.78 | 1.76 | 6.7 | 0.2 |
| Q | 14.44 | 0.1 | 0.3 | 5.74 | 4.36 |
| R | 19.2 | 0.1 | 0.3 | 4.58 | 14.56 |
| S | 5.82 | 0.1 | 0.3 | 3.46 | 0.2 |
| T | 10.38 | 0.1 | 0.3 | 5.44 | 20.18 |
| U | 9.2 | 0.1 | 0.3 | 5.76 | 2.12 |
| V | 1.78 | 0.1 | 0.3 | 6.6 | 0.2 |
| W | 4.16 | 0.1 | 0.3 | 3.24 | 0.2 |
| X | 4.64 | 0.7 | 0.3 | 7.04 | 0.2 |
| Y | 4.68 | 0.1 | 0.3 | 7.46 | 0.2 |
| Z | 3.36 | 0.1 | 0.3 | 3.08 | 0.2 |
| 1 | 5.5 | 0.1 | 0.84 | 3.76 | 0.2 |
| 2 | 10.7 | 0.1 | 0.3 | 3.2 | 0.2 |
| 3 | 3.52 | 0.1 | 0.3 | 2.06 | 3.8 |
| 4 | 1.52 | 0.1 | 0.68 | 12.82 | 12.44 |

## Results and Discussion

In the used system, the following metals could be determined: Fe(II) – retention time about 5 min, Cu(II) – 6.3 min, Ni(II) – 7.1 min, Zn(II) – 8.1 min, Co(II) – 9 min, Cd(II) – 10.5 min, and Mn(II) – 11 min. Cadmium and cobalt were not present in the sample with detectable amounts. There is also an unidentified ion eluting just before manganese, which in the case of one water forms with manganese a peak overlap.

The unprocessed ion chromatograms are shown in Fig. 1A. The PCA comparison of unprocessed data (Fig. 1B) shows outlying samples (waters B, I, R and T). Waters R and T have very high value of PC1, whereas B and I have high PC2. Analysis of loading plot (Fig. 1C) led to the conclusion that the reason of outliance is the very high content of iron and manganese (R and T) and zinc (B and I). As these waters can affect (as outliers) whole PCA computations, they were removed from the dataset before further processing.

The next step was to cut out the first part, where no interesting data is collected (only the meaningless peak of void volume). This was done in a place indicated by a dashed line in Fig. 1A. The resulting dataset is presented analogously with its PCA in Fig. 2. It can be clearly seen that samples are scattered because the significant source of variance is the variation of the baseline. The baseline drift is present also in loadings (Fig 2C), where the baselines are non-negative.

Therefore, the next processing steps were performed. To filter the data from the noise, a Savitzky-Golay filter was used with optimization against the noise correlation [17].

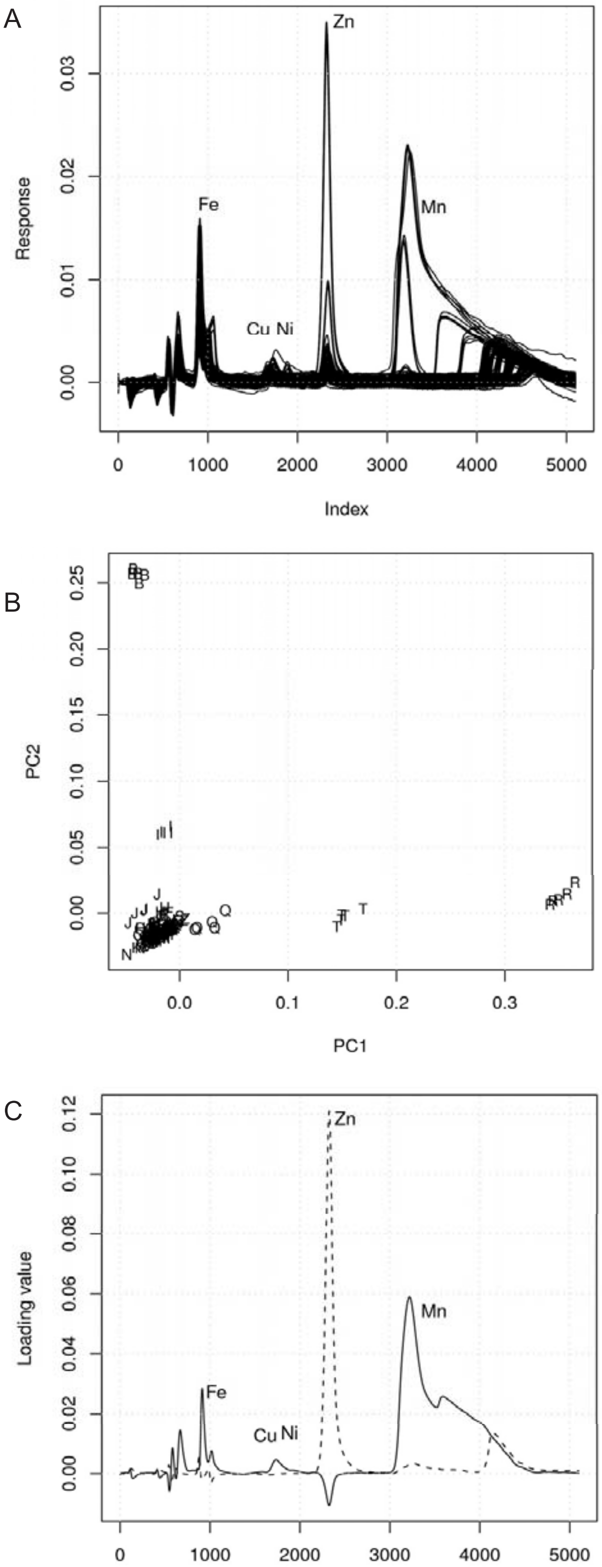

Fig. 1. The unprocessed ion chromatograms (A), their principal component analysis (B, for letters see text) and loading plots (C).

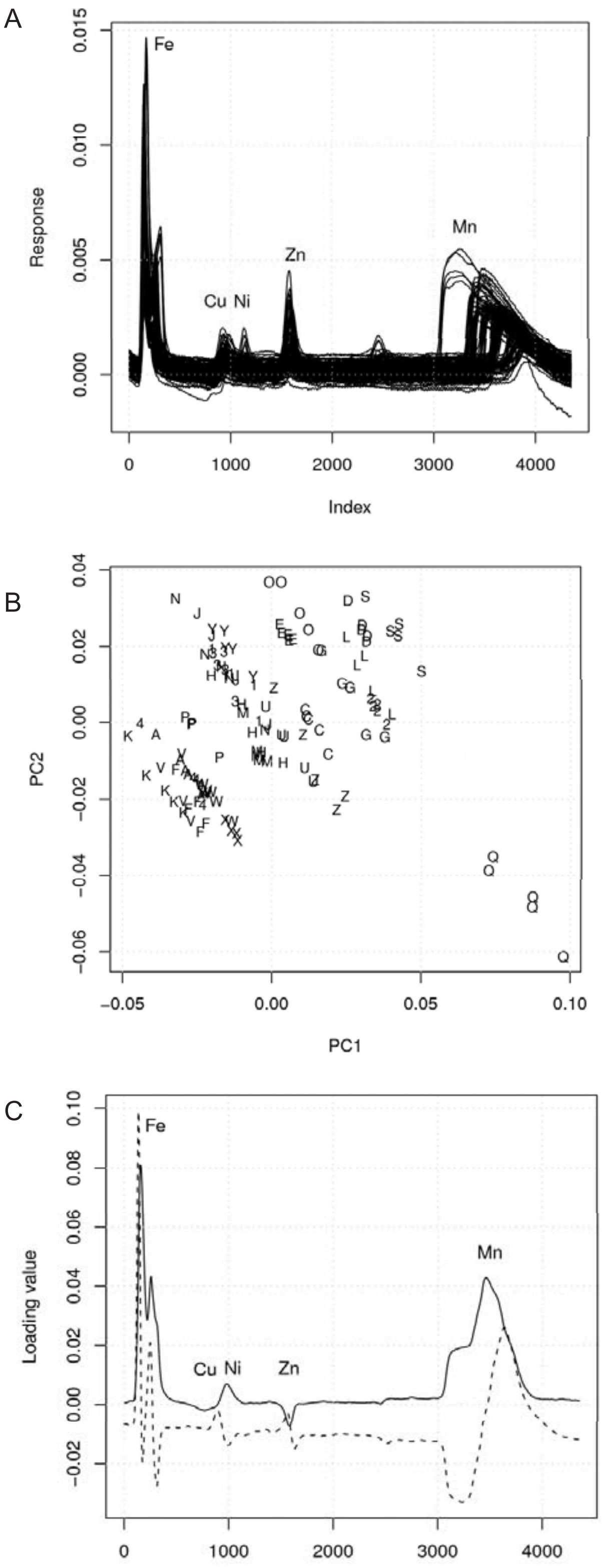

Fig. 2. Chromatograms after cutting out the void peak and removing outliers (A), their PCA analysis (B, for letters see text) and corresponding loadings (C).

This step is very important and makes baseline estimation correct without any error due to unsmoothness. The baseline was then extracted and removed [15]. Analyzing the resulting data and its PCA analysis (Fig. 3), one can conclude that the samples of the same water are located very closely. The warping procedure was not used in this study due to the following reasons [21]: a difficulty with choosing a target profile and varying position of the manganese peak. Some experiments with correlation-optimized warping (COW) were performed, but they improved neither the PCA plots nor loading shapes. Moreover, one cannot be sure if the unknown ion, present in outlying (and previously removed) samples, is not present here, shifting the peaks claiming to be manganese.

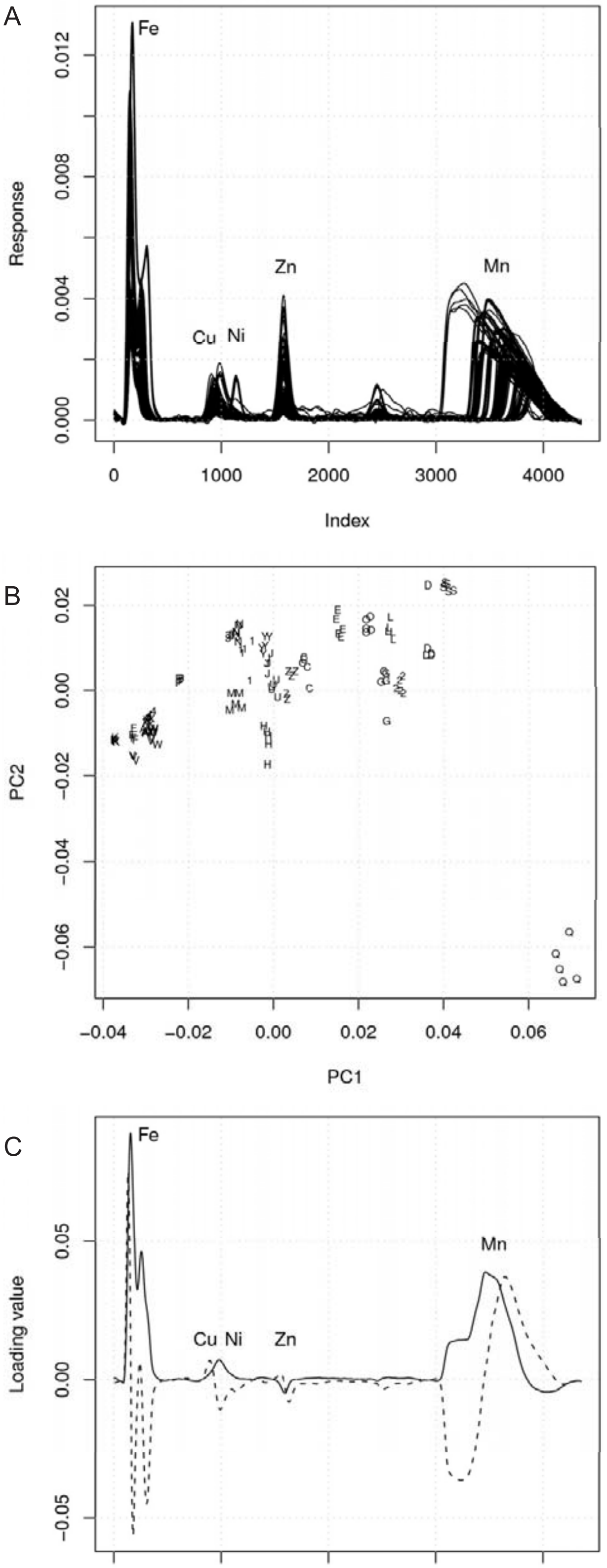

Fig. 3. The chromatograms after baseline removal (A), their PCA analysis (B, for letters see text) and loading plots (C).

Analyzing the final comparison and its loadings (Figs. 3B and 3C), one can conclude that iron, nickel, and manganese affects PC1 positively, whereas zinc affects it negatively, similar to Figs. 1C and 2C. Therefore, one can conclude that zinc concentration is correlated negatively with iron and nickel, as PC1 models 79.6% of total variance. PC2 models 8.2% of additional variance and contains mainly the information about peak shape differences. This result is similar to the real correlation between the ion content, calculated classically from peak areas (Fig. 4).

The water Q (Muszyna) is different from other groups due to high manganese and zinc content. The other waters do not form any clear and visible cluster. The waters located mostly left (low PC1 value: K – Górska Natura, F – Babia Góra and V – Primavera) contain highest concentrations of zinc and lowest concentations of other metals. The right side of the cloud (D – Aquarel, S – Nałęczowianka, 2 – Vita) have more reach in other metal ions, whereas concentrations of zinc are generally lower. The samples of the same water are clustered together, which indicates that variance related to baseline differences was removed.

## Conclusions

The chemometric processing of ion chromatograms is an interesting and fast alternative to classical integration of the peaks of particular ions. The advantage of the approach is no need to perform a routine calibration and manual

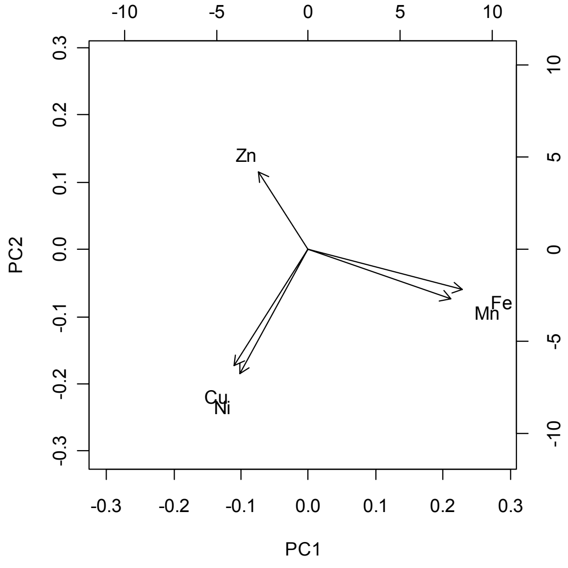

Fig. 4. The correlation between classically determined (from peak areas) ion concentrations in analyzed waters.

evaluation of the peaks. Although the multivariate comparison is affected by sensitivity differences (the same peak heights of two ions do not imply the same concentrations), it can be neglected for fast screening purposes. Denoising, baseline extraction, warping, and whole multivariate analysis can be performed automatically.

## References

1. SZYCZEWSKI P., SIEPAK J., NIEDZIELSKI P., SOBCZYŃSKI T. Research on Heavy Metals in Poland. Pol. J. Environ. Stud. **18**, 755, **2009**.
2. PESAVENTO M., ALBERTI G., BIESUZ R. Analytical methods for determination of free metal ion concentration, labile species fraction and metal complexation capacity of environmental waters: A review. Anal. Chim. Acta **631**, 129, **2009**.
3. TYAGI P., BUDDHI D., CHOUDHARY R., SAWHNEY R.L. Degradation of ground water quality due to heavy metals in industrial areas of India – A review. Ind. J. Environ. Prot. **20**, 174, **2000**.
4. ALAM I.A., SADIQ M. Metal contamination of drinking water from corrosion of distribution pipes. Environ. Poll. **57**, 167, **1989**.
5. SHARPE W.E., DEWALLE D.R. Potential health implications for acid precipitation, corrosion, and metals contamination of drinking water. Environ. Health Persp. **63**, 71, **1985**.
6. WARDAS W., HŁAWICZKA S., PRZYBYŁOWICZ I., WICHARY M. Nitrate and nitrite content in mineral table waters, sodas and soft drinks. Roczn. PZH **32**, 211, **1981**.
7. SZYDŁOWSKA E., ZARĘBA S., SZYDŁOWSKI W. The content of nitrates III and V in mineral table water and in medicinal waters. Przegl. Lek. **62**, 86, **2005**.
8. MASŁOWSKA J., GAWŁOSKA A. Determination of selenium in mineral waters by the ASA method. Roczn. PZH **50**, 261, **1999**.
9. KOT A. Determination of content of zinc and cooper in table and therapeutic mineral water. Przegl. Lek. **7**, 14, **2001**.
10. GRAZULEVICIENE R., NADISAUSKIENE R., BUINAUSKIENE J., GRAZULEVICIUS T. Effects of elevated levels of manganese and iron in drinking water on birth outcomes. Pol. J. Environ. Stud. **18**, 819, **2009**.
11. MICHALIK A. The use of chemical and cluster analysis for studying spring water quality in Świetokrzyski National Park. Pol. J. Environ. Stud., **17**, 357, **2008**.
12. MICHALSKI R. Ion chromatography method for the determination of trace levels of chromium(VI) in water. Pol. J. Environ. Stud., **13**, 73, **2004**.
13. ŚWIĘCICKA D., GARBOŚ S. Investigation of concentration levels of chromium(VI) in bottled mineral and spring waters by high performance ion chromatography technique with application of postcolumn reaction with 1,5-diphenylcarbazide and VIS detection. Roczn. PZH. **59**, 397, **2008**.
14. FELLINGER A. Data Analysis and Signal Processing in Chromatography, Elsevier, Amsterdam, **1998**.
15. DASZYKOWSKI M., WALCZAK B. Use and abuse of chemometrics in chromatography. Trends Anal. Chem. **25**, 1081, **2006**.
16. PASTI L., WALCZAK B., MASSART D.L., RESCHIGLIAN P. Optimization of signal denoising in wavelet transform (DWT). Chemom. Intell. Lab. Syst. **48**, 21, **1999**.
17. KOMSTA Ł. A comparative study on several algorithms for denoising of thin layer densitograms. Anal. Chim. Acta, **641**, 52, **2009**.
18. PRAVDOVA V., WALCZAK B., MASSART D.L. A comparison of two algorithms for warping of analytical signals, Anal. Chim. Acta, **456**, 77, **2002**.
19. WALCZAK B., WU W. Fuzzy warping of chromatograms, Chemom. Intell. Lab. Syst., **77**, 173, **2005**.
20. STANIMIROVA I., DASZYKOWSKI M., WALCZAK B. Dealing with missing values and outliers in principal component analysis, Talanta, **72**, 172, **2007**.
21. DASZYKOWSKI M., WALCZAK B. Target selection for alignment of chromatographic signals obtained using monochannel detectors, J. Chromatogr. A, **1176**, 1, **2007**.