Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1084 (2005) 180–185
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Optimization of solid-phase extraction using artificial neural networks in combination with experimental design for determination of resveratrol by capillary zone electrophoresis in wines

Miroslava Spanilá*, Jiří Pazourek, Marta Farková, Josef Havel

Department of Analytical Chemistry, Faculty of Science, Masaryk University, Kotlářská 2, CZ-61137 Brno, Czech Republic

Abstract

Solid-phase extraction (SPE) is often used for preconcentration of analytes from biological samples. Such an analytical step requires optimization for obtaining reliable results. Optimization in analytical chemistry is traditionally still often done with relaxation method, when an optimal value of a single variable is searched for (single variable approach (SVA)). However, if the optimized procedure is complex, there is a danger not to find the real optimum by SVA. Therefore, more advanced optimization approaches should be applied—multivariable approach (MVA). Applying MVA optimization and finding the real optimum, better experimental conditions are obtained and thus, time, chemicals and analytical procedure cost can be served. Nowadays, using artificial neural networks (ANN's) in combination with MVA is rapidly expanding. In this work, the optimization of SPE using relaxation method (SVA) and optimization by ANN's in combination with experimental design (MVA) are compared and latter approach is practically illustrated. Advantages of MVA over SVA for optimization are discussed. The prediction of the optimal SPE conditions for determination *cis*- and *trans*-resveratrol in Australian wines by capillary zone electrophoresis is described and the improvement of efficiency of SPE using MVA is confirmed.

Keywords: Solid-phase extraction; Artificial neural networks; Experimental design; Single variable approach; Multivariable approach; Capillary electrophoresis; Resveratrol

1. Introduction

Each analytical method consists of specific sets of experimental conditions. To find the best conditions, optimization is usually required. One kind of optimization strategies is based on some assumptions about the experimental space (a systematic scan of the space and the random search by experimental design). Other strategies make strong assumptions about the response surface. They try to find the optimum as quickly as possible and search only in a local area of the search space (Simplex) [1].

The single variable approach (SVA) or multivariable approach (MVA) are commonly used for optimization of analytical methods. The first one may require many experiments and thus much experimental work. In this "step-by-step" approach, one experimental parameter is regularly changed within an interval of interest while others parameters are kept constant. When a presumptive optimum of the tested parameter is found, the value is fixed and another parameter is to be optimized. Testing every possible points of each parameter is time and cost consuming, which is serious, and may not lead to an optimum at all. The mistake can occur when a local optimum instead of a global one is found.

In the second approach, all experimental parameters are changed simultaneously and the probability of global optimum finding is much higher. This goal is effectively gained by MVA. To prepare an experimental set which ensures the maximal information about the data set, a suitable experimental design should be used.

* Corresponding author. Tel.: +420 54949 6211; fax: +420 54121 1214.
E-mail address: spmirka@mail.muni.cz (M. Spanilá).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.10.007

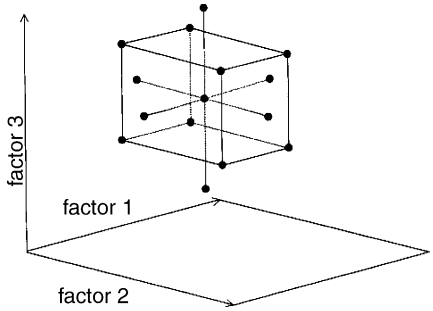

Fig. 1. General scheme of the three-level central composite design (CCD). The points belongs to apportionment of experiments over intervals of optimized parameters (e.g. factor 1, volume of sample; factor 2, the flow rate; and factor 3, volume of eluent).

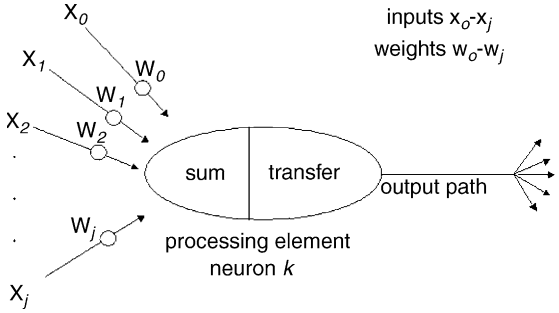

Fig. 2. Basic element of ANN (neuron k), receiving inputs data from neighbouring neurons x_0–x_i (with weights w_0–w_j), processing of information (for sum see Eq. (1)) and dispatching output data to other neuron.

1.1. Experimental design

By an experimental design (ED), a planned series of operations called experiments is meant. When more than one variable is changed between experiments the individual conditions are called factors. The particular values at which experiments are run are called the factor levels. ED is applied to determine by an efficient informative way the set of conditions that are required to obtain a product or process with desirable, often optimal characteristics [1].

An unlimited number of experimental design composites is possible. Including different patterns in different parts of search space, each is designed to efficiently answer a particular question. For systematic optimization, central composite design (CCD) is frequently used (Fig. 1).

CCD is very efficient, providing much information on experiment variable effects and overall experimental error in a minimum number of required runs. In comparison with SVA, in multivariate approach, the analysis time and number of experiments is reduced and statistical interpretation possibilities are increased. The acquired data from experimental design are evaluated using chemometrical methods. Applications of experimental designs in combination with artificial neural networks (CCD-ANN) for capillary zone electrophoresis has been first time applied by Havel and cowrkers [2]. Later, a various applications of ANN's for chemical data evaluation has been done [3–9].

1.2. Artificial neural networks

Application of ANN's for data processing is characterized by a simplified analogy with biological neurons. Each neuron is linked to certain of its neighbours with varying coefficients of connectivity that represent the strengths (the weights) of these connections (Fig. 2). A network of artificial neurons is composed of a large number of simple, highly interconnected processing elements (neurons) working in parallel providing an output response to an input data [10].

The neurons are sorted in an input layer, hidden layer(s) (one or more) and an output layer (notation I:H:O). Input neurons accept the input data characterizing each observation, output neurons provide the predicted value or pattern, and hidden neurons neither receive inputs directly nor provide output values directly. The neuron sums the product of each connection weight (w_{jk}) from a neuron j to the neuron k and input (x_j) and the additional weight called the bias to get the value sum for the neuron k (Eq. (1)).

$$\text{sum}_k = \sum_j x_j w_{jk} + \text{bias}_k \quad (1)$$

The sum of the weighted inputs is transformed with a transfer function and this function is used to get the output level. A process of training means that the weights are corrected so as to produce prespecified ("correct") target values. The training requires sets of pairs (X_S, Y_S) for input: the actual input into the network is the vector X_S, and the corresponding target, or prespecified answer, is labelled Y_S. The goal of the training is to correct the weights that will give the correct answers Y_S for each vector X_S from the training set. After the training has been completed successfully, it is hoped that the network will give correct predictions for any new object X. As the training method of multilayered neural network, back propagation was used [11]. Finally, after the learning phase using mathematical algorithm, ANN can predict desired information.

The theory of different networks has been reviewed by Zupan and Gasteiger [12]. In this work, a multilayered feed-forward neural network was used. As the learning scheme, the algorithm of back-propagation in combination with quick propagation, which attempts to use a simple quadratic model of the error surface (calculated independently along each weight) to speed convergence, was applied.

1.3. Solid-phase extraction

Solid-phase extraction (SPE) is commonly used as a clean up and preconcentration technique. A liquid sample is passed through the column, which is filled with a polymeric porous sorbent. The sample compounds with chemical affinity to sorbent are retained and consecutively eluted by suitable solvent. The extraction is a complex process and according to the character of the sample (polarity, solubility, pK_a, . . .) the sorbent

and elution solvent should be carefully chosen. Instead of the flow rate or temperature, the amount of sample and elution solvent composition are the most important factors for efficiency of extraction. Therefore, the optimization of the important factors is required for the real optimum searching.

The versatility of SPE allows it to be used for purification, trace enrichment, solvent exchange, etc. In this work, SPE has been used for preconcentration of resveratrol in wines [13–16]. To our knowledge known method using C_{18} cartridge for extraction of resveratrol was taken [13] and because of complexity of this method, the optimization using relaxation method and ED-ANN's was done. The optimized parameters were: the flow rate of solutions though the column, the volume of the sample and the volume of the eluent. The optimal conditions were taken for electrophoretic analysis.

1.4. Capillary electrophoresis

Capillary electrophoresis is current method used for rapid analysis of biological and environmental samples. The separation of analytes is based on different mobilities of analytes in electric field [19]. The preconcentration of wines by SPE is recommended to obtain lower detection limit and to eliminate the interferences of other compounds presented in wines [13–18]. In this work, the electrophoretic separation conditions presented by Pazourek and coworkers [13] were used for analyses of samples of Australian wines. The determination of *cis*- and *trans*-resveratrol was carried out.

The main goal of this paper was reach the higher efficiency for resveratrol extraction. The optimal conditions for SPE of resveratrol from Australian wine samples proposed by relaxation method and by central composite design for three factors in combination with artificial neural network were compared. The optimal conditions with the highest efficiency were taken for electrophoretic analysis of wine samples and for determination and quantification of *cis*- and *trans*-resveratrol in the samples.

2. Experimental

2.1. Materials and instrumentation

The extraction columns BakerBond SPE octadecyl (C_{18}) endcapped reversed phase by J.T. Baker (Phillipsburg, NJ, USA) with 100 mg of octadecyl and 1 ml column size were used (product number 7020-01). The solutions were dosed by peristaltic pump Labeco PCR 01 (Spišská Nová Ves, Slovakia) on the column. The dosing vessel, the SPE column and the detector were connected with tubes i.d. 0.032 from Gilson. The spectrophotometric detector HP 1050 connected with liner recorder TZ 4620 from Laboratory Instruments (Prague, Czech Republic) was used. The measurements were done at room temperature and the response of detector was detected at 305 nm.

2.2. Chemicals

The standard solution of resveratrol from Sigma (St. Louis, Mo, USA), 0.1 mM concentration was prepared in 12% ethanolic solution. Methanol (99.8%), ethanol (>99%) and sodium tetraborate from Lachema (Brno, Czech Republic) were purchased. Mesityloxide from Fluka (Buchs, Switzerland) for EOF measurement and sodium hydroxide from Merci (Brno, Czech Republic) was used. Water was double distilled in Heraeus apparatus (Hanau, Germany).

2.3. Software

The data were processed using Trajan 3.0 software package (Trajan Neural Network Simulator, Release 3.0 D, Copyright Trajan Software Ltd. (1996–1998)).

2.4. Capillary electrophoresis

Electrophoretic measurements were carried out using 3D CE Agilent Technologies equipment provided with a diode array detector and fused-silica capillary (Composite Metal Services, The Chase, Hallow, UK), total length 38.5 cm (effective length 30 cm) × 75 μm i.d. As the background electrolyte, 25 mM borate pH 9.38 was used. The sample was hydrodynamically injected for 4 s with 50 mbar and the positive separation voltage +20 kV was applied. The EOF has been controlled by measuring of 0.1% mesityloxide. All the measurements were measured at 25 °C and were collected at 305 nm.

The capillary was first conditioned with 1 M sodium hydroxide for 30 min at 40 °C and flushed 10 min with double distilled water and 10 min with the 25 mM borate running buffer. Between the analysis, the capillary was flushed with 0.1 M sodium hydroxide (1 min), water (1 min), and running buffer (2 min).

3. Results and discussion

3.1. SVA

Single variable approach optimization was spectrophotometricaly measured as first. Standard solution of resveratrol 0.1 mM used for extraction was prepared in 12% ethanolic solution to simulate matrix of wine. The parameters were the flow rate (y), the sample volume (x) and the volume of eluent (z). The other parameters were kept constant. The column was treated in following procedure: 2 ml of methanol for preconditioning, 2 ml of distilled water to clean off the impurities, x ml of sample solution, $2x$ ml distilled water for matrix flushing and finally z ml of methanol for analyte elution. In the first step, the flow rate was changed within the range 0.3–1.2 ml min^{-1}. The sample volume and the methanol volume for elution was 1 ml. Observed data were evaluated manually. Half-width $w_{1/2}$ and retention time t_m

Table 1
Experimental design for SPE optimization

Run	Volume of sample x (ml)	Flow rate y (ml min^{-1})	Volume of methanol z (ml)	Efficiency[a] N_{calc}
1	0.80	0.50	1.60	11.9
2	0.80	0.50	4.00	10.1
3	0.80	1.00	1.60	10.8
4	0.80	1.00	4.00	8.8
5	2.00	0.50	1.60	10.9
6	2.00	0.50	4.00	8.5
7	2.00	1.00	1.60	8.5
8	2.00	1.00	4.00	7.9
9	1.40	0.75	2.80	11.5
10	0.35	0.75	2.80	11.1
11	2.45	0.75	2.80	8.4
12	1.40	0.30	2.80	7.6
13	1.40	1.20	2.80	7.9
14	1.40	0.75	0.68	9.4
15	1.40	0.75	4.92	8.3
16	1.40	0.75	2.80	11.5
17	0.50	0.60	3.00	11.2
18	1.60	1.05	1.70	8.4
19	1.10	0.45	1.00	11.9

[a] Efficiency $N = 5.54\,(t_m/w_{1/2})^2$; t_m, retention time of the peak; $w_{1/2}$, half width of the peak.

of each recorded peak was measured and the efficiency N was calculated using standard equation (Table 1). In the second step, the volume of sample was optimized in the range 0.3–2.5 ml and finally the methanol volume was optimized in the range 0.6–5 ml and efficiency N was calculated. The optimal conditions found by SVA were: 1.1 ml of the sample, the flow rate 1 ml min^{-1} and 2 ml of methanol for elution. The number of experiments was 45. The recovery of resveratrol was 90%.

3.2. MVA

Multivariable optimization approach requires all the variables changed in the same time. To do this an experimental design is usually applied. In our case, we applied central composite design for three factors (parameters) (Fig. 1). Each parameter was tested at five levels. The number of 16 experiments was performed. The variables x, y and z and calculated efficiencies N_{calc} are shown in the table (Table 2).

Table 2
Calculated and predicted efficiency (outputs of ANN)

Run	Efficiency N_{calc}[a]	Efficiency N_{pred}[b]	$N_{calc} - N_{pred}$	Error	Error relative $N_{calc} - N_{pred}$ (%)
1	11.9	12.5	−0.6	0.11	−4.43
2	10.1	10.5	−0.4	0.09	−4.03
3	10.8	10.9	−0.1	0.01	−0.51
4	8.8	9.2	−0.4	0.09	−4.72
5	10.9	10.9	−0.0	0.00	−0.22
6	8.5	8.6	−0.1	0.02	−1.14
7	8.5	8.4	0.1	0.03	1.76
8	7.9	7.6	0.3	0.02	1.47
9	11.5	11.6	−0.1	0.01	−0.54
10	11.1	10.6	0.5	0.11	5.14
11	8.4	8.6	−0.2	0.04	−2.20
12	7.6	7.6	0.0	0.00	0.02
13	7.9	8.2	−0.3	0.05	−2.77
14	9.4	9.4	−0.0	0.00	−0.15
15	8.3	8.3	−0.0	0.00	−0.06
16	11.5	11.7	−0.2	0.03	−1.31
17	11.2	11.2	−0.0	0.01	−0.37
18	8.4	8.9	−0.5	0.09	−4.84
19	11.9	12.6	−0.7	0.12	−4.77

1–16, training values and 17–19, values of verification.
[a] $N_{calc} = 5.54\,(t_m/w_{1/2})^2$; t_m, retention time of the peak; $w_{1/2}$, half width of the peak.
[b] N_{pred}, prediction ANN's (3:3:1).

Table 3
Comparison of SVA and MVA optimal conditions

	Volume of sample (ml)	Flow rate (ml min^{-1})	Volume of methanol (ml)	Efficiency N_{calc}[a]
SVA	1.10	1.00	2.00	11.1
MVA	0.85	0.40	0.75	13.0

[a] $N_{calc} = 5.54\,(t_m/w_{1/2})^2$; t_m, retention time of the peak; $w_{1/2}$, half width of the peak.

The data obtained from experimental measurements were used for modelling using artificial neural networks. The variables were used as inputs for ANN, as output the value of efficiency of extraction was used.

The data set of experimental design was divided onto 16 values of the training set and 3 values were used for the verification (15 training values were taken on the ground of experimental design and the central point was measured twice). The verification values were randomly taken from the ranges of all optimizing variables.

Table 4
Analysis of wines—determination of *cis*-resveratrol and *trans*-resveratrol

Wine	Producer, year and denomination of origin	*cis*-Resveratrol (mgl^{-1})	*trans*-Resveratrol (mgl^{-1})
White wines	Lombard Station, Chardonnay 2001, Riverina Estate, Riverina Wine, Griffith, Australia	0.76	1.07
	Three corners, Chardonnay 2001, Riverina Estate, Riverina Wine, Griffith, Australia	0.36	1.98
Red wines	Bushman's Gully, Cabernet Merlot, 2001, Riverina Estate, Riverina Wine, Griffith, Australia	1.62	3.71
	Bushman's Gully, Shiraz Cabernet, 2001, Riverina Estate, Riverina Wine, Griffith, Australia	1.81	4.46
	Bushman's Gully, Cabernet Sauvignon, 2001, Riverina Estate, Riverina Wine, Griffith, Australia	1.03	2.11
	Lombard Station, Cabernet Sauvignon, 2001, Riverina Estate, Riverina Wine, Griffith, Australia	1.32	2.98
	Three corners, Shiraz, 2001, Riverina Estate, Riverina Wine, Griffith, Australia	1.31	2.92

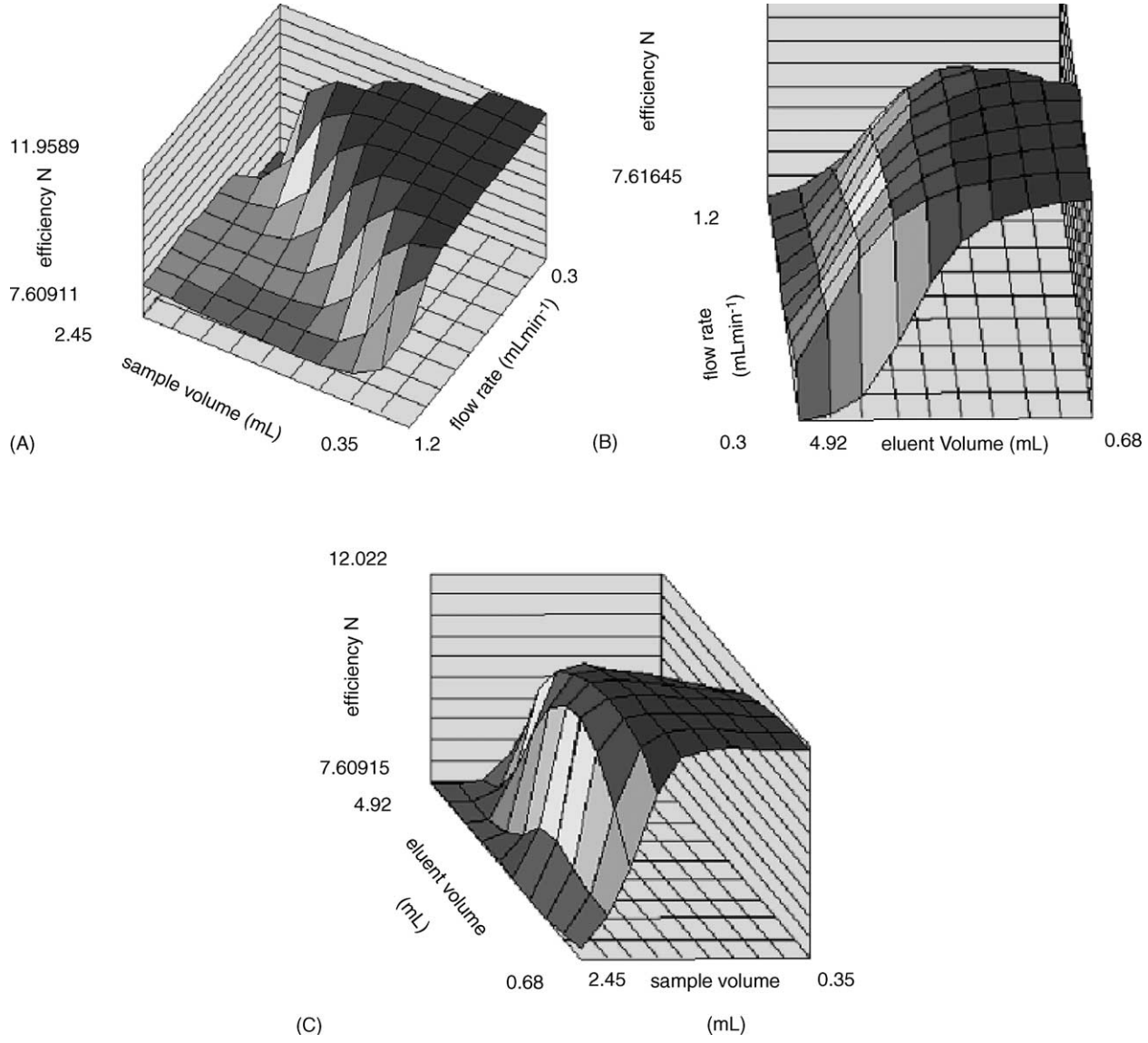

Fig. 3. The graphs of the response surface. (A) Efficiency as the function of the sample volume x (0.35–2.45 ml) and the flow rate y (0.3–1.2 ml). (B) Efficiency as the function of the flow rate y (0.3–1.2 ml) and the eluent volume z (0.68–4.92 ml). (C) Efficiency as the function of the eluent volume z (0.68–4.92 ml) and the sample volume x (0.35–2.45 ml).

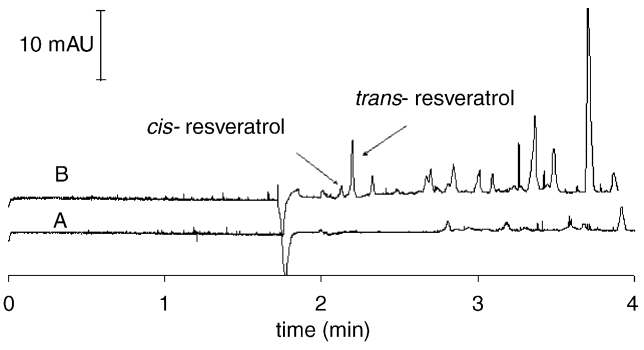

Fig. 4. Electropherograms of white wine. Running buffer 25 mM tetraborate, pH 9.38, separation voltage +20 kV, hydrodynamic injection 4 s (50 mbar), temperature 25 °C, detection wavelength 305 nm. (A) Direct injection of wine and (B) preconcetration using SPE.

Back propagation in combination with quick propagation as a training algorithm for multilayer perceptrons was applied for the suitable network searching. During the training process, the variable values of weights between individual neurons were assigned. Simultaneously, the values of error, used to determine how a neural network is performing during iterative training and execution, were calculated. The ANN architecture was searched for by the Trajan Neural Network Simulator. The optimal network was afterwards used for the training process and the prediction, as well.

The program Trajan tested neural networks with one to seven neurons in the hidden layer with 1000 epochs for training and the optimal structure of the network with three neurons in the hidden layer was applied for further prediction (3:3:1). This network predicted efficiency with the error up to 5% (Table 2) and on this base, the optimal conditions

were found: 0.85 ml of sample, 0.40 ml min^{-1} flow rate and 0.75 ml of eluent.

For the better view of given results, the graphs of the response surface were drawn. The best conditions for the maximum efficiency can be seen in the graph of the response surface for each couple of tested parameters. There is the response surface of the sample volume and the flow rate (Fig. 3A), the flow rate and the volume of eluent (Fig. 3B) and the volume of eluent and the volume of sample (Fig. 3C). In the graphs it is clearly shown, that lower flow rate, lower eluent volume and lower volume of sample gives higher efficiency. Finally, the results obtained by SVA and MVA optimization are compared (Table 3).

3.3. Applications of the optimized conditions

SPE is useful preconcentration method in analysis of wine. In direct injection of wine (without preconcentration), both forms of the resveratrol are under the limit of detection (Fig. 4). In CZE of Australian wine samples, the optimal conditions of SPE found by MVA were applied The LOD of *trans*-resveratrol was 0.26 mgl^{-1} (for preconcentrated samples). The quantification of *cis*- and *trans*-resveratrol in the samples was done (Table 4).

4. Conclusions

The advantages of multivariable approach were shown. The number of experiments using CCD-ANN's was more than two times lower and consecutively the time of experiment was greatly reduced using multivariable approach. Moreover, the solvent and sample consumption was reduced too. It was shown, that optimization using ED-ANN's is faster and save-cost in comparison with relaxation method (SVA) and the optimal conditions found by CCD-ANN's were used for *cis*- and *trans*-resveratrol determination in Australian wines. The limit of detection of *trans*-resveratrol was 0.26 mgl^{-1} (for preconcentrated samples).

Acknowledgement

This work was supported by project 203/02/1103 of Grant Agency of the Czech Republic.

References

[1] D.L. Massart, B.G.M. Vandeginste, L.M.C. Buydens, S. de Jong, P.J. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics: Part A, Elsevier Science B.V., Amsterdam, The Netherlands, 1997.
[2] J. Havliš, J.E. Madden, A.L. Revilla, J. Havel, J. Chromatogr. B 755 (2001) 185.
[3] J. Havel, E.M. Peña-Méndez, A. Rojas-Hernández, J.-P. Doucet, A. Panaye, J. Chromatogr. A 793 (1998) 317.
[4] L. Pokorná, A.L. Revilla, J. Havel, J. Patočka, Electrophoresis 20 (1999) 1993.
[5] J. Havel, J.E. Madden, P.R. Haddad, Chromatographia 4 (1999) 481.
[6] M. Farková, E.M. Peña-Méndez, J. Havel, J. Chromatogr. A 848 (1999) 365.
[7] V. Dohnal, M. Farková, J. Havel, Chirality 11 (1999) 616.
[8] J. Havel, J.E. Madden, P.R. Haddad, Chromatographia 49 (1999) 481.
[9] J. Havel, M. Breadmore, M. Macka, P.R. Haddad, J. Chromatogr. A 850 (1999) 345.
[10] V. Kvasnička, Š. Sklenák, J. Pospíchal, Chem. Listy 87 (1993) 79.
[11] Trajan Neural Network Simulator, Release 3.0 D, Copyright Trajan Software Ltd, 1996–1998.
[12] J. Zupan, J. Gasteiger, Neural Networks in Chemistry and Drug Design, WILEY-VCH Verlag Gmbh, Weinheim, Federal Republic of Germany, 1999.
[13] Z. Dobiášová, J. Pazourek, J. Havel, Electrophoresis 23 (2002) 263.
[14] D. Martínez, M.J. Cugat, F. Borull, M. Calull, J. Chromatogr. A 902 (2000) 65.
[15] X. Gu, Q. Chu, M. O'Dwyer, M. Zeece, J. Chromatogr. A 881 (2000) 471.
[16] J. Pazourek, G. Gonzáles, A.L. Revilla, J. Havel, J. Chromatogr. A 874 (2000) 111.
[17] L. Arce, M.T. Tena, A. Rios, M. Valcárcel, Anal. Chim. Acta 359 (1998) 27.
[18] M. Vaher, M. Koel, J. Chromatogr. A 990 (2003) 225.
[19] J.W. Jorgenson, K.D. Lukacs, Science 222 (1983) 266.