Application of Doehlert matrix and factorial designs in the optimization of experimental variables associated with the on-line preconcentration and determination of zinc by flow injection inductively coupled plasma atomic emission spectrometry

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A simple and rapid method is described for the preconcentration and determination of zinc in waters. The method employs on-line preconcentration of zinc on a 3 cm \times 3 mm column packed with silica gel functionalized with 1,5-bis(di-2-pyridyl)methylene thiocarbohydrazide (DPTH-gel) and placed in the injection valve of a simple flow manifold. Five variables (sample flow rate, eluent flow rate, eluent concentration, pH and buffer concentration) were considered as factors in the optimization process. Interactions between analytical factors and their optimal levels were investigated using two level factorial and Doehlert matrix designs. The optimum conditions established were applied to the determination of zinc by flow injection inductively coupled plasma atomic emission spectrometry. The proposed method has a linear calibration range from 2 to at least 500 ng ml\(^{-1}\) of zinc, with a detection limit of 1.7 ng ml\(^{-1}\) (S/N=3) and a throughput of 40 samples h\(^{-1}\) using a 60 s preconcentration time. Validation was carried out against certified reference water samples, and by determining the analyte content in sea water and synthetic sea water. The results showed good agreement with the certified values and sufficiently high recoveries.

Introduction

The determination of heavy metals is required for the routine monitoring of marine environmental pollution. Although electrothermal atomic absorption spectrometry (ET-AAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) have very low detection limits for trace metals in aqueous solution, the direct determination of trace metals in sea water by these techniques is difficult even with sophisticated background correction and chemical modification. This is due to the low concentrations and strong interference from the sample matrix. ETAAS or ICP-AES with on-line sorbent extraction separation and preconcentration can solve the two problems mentioned above and lead to easy determination. This operation can be performed in a flow injection (FI) system by coupling flow injection on-line to a conventional separation unit, such as a mini-column packed with a suitable adsorbent or ion-exchange material, or a liquid–liquid extraction unit, before the detection system. Some on-line column preconcentration manifolds for ICP-AES have been reported. Thus, Hartenstein et al.\(^8\) described a flow injection (FI)-ICP method utilizing an ion-exchange column of Chelex-100 to increase the sensitivity for multielement measurements. This method gives simultaneous multielement detection limits which are over 20 times better than those obtained with conventional continuously aspirated systems for Ba, Be, Cd, Co, Cu, Mn, Ni and Pb. Hirata et al.\(^5\) used Muramoc A-1 chelating resin for on-line column preconcentration coupled with ICP-AES. Activated alumina has been used for adsorption of oxyanions and Mo. The determination of 20 elements was proposed by Wang and Barnes,\(^6\) who assessed the performance of two resins for the determination of Cu and Zn in natural and drinking water. Porta et al.\(^7\) described an on-line preconcentration method utilizing an Amberlite XAD-2 resin functionalized with 1-(2-thiazolylazo)-2-naphthol to preconcentrate Cd, Cu, Fe, Mn, Ni and Zn from river water and sea water prior to their determination by ICP-AES; the detection limits ranged from 8 to 40 ng ml\(^{-1}\).

In developing a FI preconcentration and matrix separation on solid sorbents procedure, one needs to adjust many variables (e.g., sample flow rate, eluent flow rate, eluent concentration, pH and buffer concentration) in order to establish optimum conditions for the analysis. This can be very time-consuming if a conventional univariate optimization is undertaken manually and, where interactions exist between the variables, one is unlikely to find the true optimum.\(^10\) The problem is greater if the optimization procedure needs to be in accordance with the quality policy of a particular laboratory. A fruitful way to conduct experimental designs and optimization is according to surface-response methodology (SRM), which includes a group of mathematical-statistical techniques that were designed to optimize the analytical response by producing a model in which a response function corresponds to several variables called factors. To carry out an optimization with a multivariate method, experimental designs must be used. Basically, such designs are of two types: primary order designs in which the equation is defined as

\[
Y = a + bA + cB + dB + dAB
\]

and second order designs in which the equation is:

\[
Y = a + bA + cB + dA^2 + eB^2 + fAB
\]

where \(Y\) is the experimental response, \(A\) and \(B\) represent the variables to be optimized, \(a\) is an independent term, \(b\) and \(c\) represent coefficients of the linear terms, \(d\) and \(e\) are the coefficients of the quadratic terms and \(f\) is the coefficient of the interaction term.

The second order types provide more and better information than the primary order types because they not only determine the influence of the variables to be optimized on the response, but also enable the response function to be obtained and

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was optimized by resorting to two level factorial designs and Doehlert matrix designs, using peak height as the analytical signal. Five experimental variables were considered as factors in the optimization process: sample flow rate, eluent flow rate, eluent concentration, pH and buffer concentration.

**Experimental**

**Instrumentation**

The ICP-AES system used was a Perkin-Elmer 40 sequential emission spectrometer equipped with a Perkin-Elmer AS-90 autosampler and controlled by an IBM XT-486 personal computer. The spectrometer output was connected to a PE Nelson Model 1020 personal integrator. Samples were introduced *via* a Gem Tip cross-flow nebulizer (Perkin-Elmer) fitted to a Scott-type, double pass spray chamber. The plasma operating conditions used are summarized in Table 1.

A schematic diagram of the automated on-line preconcentration system modified from the Perkin-Elmer FIAS 400 is presented in Fig. 1. Pump P1 was connected to the sample capillary and the microcolumn through a four-way rotary valve; this valve had the column connected within the sample loop. The microcolumn was a glass tube (3 cm × 3 mm id), packed with the chelating resin to a height of 0.7 cm. At both ends of the microcolumn, polyethylene frits (Omnifit) were fixed to prevent material losses. Transport lines were made using 0.8 mm id PTFE tubing. The mini-column was initially flushed with 2 M nitric acid; subsequent use of the eluent in each operating cycle was sufficient to make it ready for re-use. All glassware was washed with 10% nitric acid and rinsed with deionized water immediately before use.

**Reagents and samples**

High-purity reagents were employed in all experiments. For the synthesis of DPTH-gel, the following were used: silica gel (particle size, 0.2–0.3 mm), 3-aminopropylethoxysilane and diglutaric aldehyde from Fluka (Buchs, Switzerland), thiocarbonylrazide and di-2-pyridyl ketone from Aldrich Chemie (Steinheim, Germany), and ethanol and toluene from Carlo Erba (Milan, Italy). The synthesis of this resin was described in a previous paper.29 The structure of DPTH-gel is shown in Fig. 2. A standard 1000 µg ml⁻¹ Zn(II) solution (Fluka) was used. Standards of working strength were made by appropriate dilution as required, immediately prior to use. Water was de-ionized with a Milli-Q system. Buffers solutions of required pH were prepared from 0.5 M boric acid (Fluka) and 0.5 M sodium hydroxide solutions (Carlo Erba). The certified reference materials (CRMs) analysed to determine the accuracy of the proposed procedure were: Community Bureau of Reference (BCR), CRM 505 Estuarine (particle size, 0.2–0.3 mm), 3-aminopropylethoxysilane and diglutaric aldehyde from Fluka (Buchs, Switzerland), thiocarbonylrazide and di-2-pyridyl ketone from Aldrich Chemie (Steinheim, Germany), and ethanol and toluene from Carlo Erba (Milan, Italy). The synthesis of this resin was described in a previous paper.29

### Table 1 Optimum operating conditions for the FI-ICP determination of zinc

<table>
<thead>
<tr>
<th>ICP system</th>
<th>Wavelength</th>
<th>RF generator Frequency</th>
<th>incident power</th>
<th>Photomultiplier voltage</th>
<th>Plasma gas flow rate</th>
<th>Auxiliary gas flow rate</th>
<th>Nebulizer type</th>
<th>Nebulizer gas flow rate</th>
<th>Spray chamber</th>
<th>Plasma viewing height</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>213.856 nm</td>
<td>40 MHz, incident power 1.1 kW</td>
<td></td>
<td>900 mV</td>
<td>1211 min⁻¹</td>
<td>0.61 min⁻¹</td>
<td>Cross-flow</td>
<td>0.531 min⁻¹</td>
<td>Scott-type</td>
<td>15 mm (above the induction coil)</td>
</tr>
<tr>
<td>FI system</td>
<td>Internal diameter of coils</td>
<td>0.8 mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
water and CRM 403 Sea water. The composition of the synthetic sea water (SSW) was (in g l⁻¹): 27.9 of NaCl, 1.4 of KCl, 2.8 of MgCl₂, 0.5 of NaBr and 2.0 of MgSO₄, according to the specifications of R.C. Weast,³⁰ spiked with 4 ng ml⁻¹ of Zn. Sea water was collected in polypropylene bottles previously cleaned by soaking in 2 M nitric acid. Samples were filtered by using a membrane of 0.45 μm pore size, acidified to 1% (v/v) with concentrated HNO₃ and stored frozen until analysis.

The pH of the samples was adjusted to an optimum pH with concentrated NaOH and buffer solutions and, finally, the samples were diluted to 50 ml with de-ionized water in a calibrated flask. Samples were analysed, in triplicate, immediately after preparation, by introducing them into the manifold depicted in Fig. 1.

On-line preconcentration and elution procedure

The FI manifold used for on-line preconcentration and elution is shown in Fig. 1. The FI system was operated as follows: during the 1 min sample loading period, with the valve in the “fill” position, a sample (standard or blank) at known pH buffered with boric acid–sodium borate is pumped (via pump P₁) through the microcolumn (located in the loop of the valve). The metal is adsorbed on the sorbent mini-column and the sample matrix sent to waste. During preconcentration, a flow of eluent (1% citric acid and 1% tartaric acid) is being aspirated by the pump P₂ peristaltic pumps; and W waste. For further detail see text.

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Fig. 1 Schematic diagram of FI-ICP system for the preconcentration, separation and determination of zinc: P₁ and P₂ peristaltic pumps; and W waste. For further detail see text.

Optimization strategy

The experimental variables were optimized by applying a factorial experimental design at two levels and with Doehlert designs. The experimental data were processed following the scheme in Fig. 3, making use of the STATGRAPHICS program.¹¹ The main features of Fig. 3 are described below.

Factorial design. Several variables could potentially affect the response surface: eluent flow rate, sample flow rate, eluent concentration, pH and buffer concentration. Consequently, a two level full factorial design, ²³ involving 32 runs was used as the first approximation to the response surface of the preconcentration process. Table 2 list the upper and lower values given to each factor and Table 3 shows the experimental design matrix and the results obtained in each run.

Fig. 2 Structure of DPTH-gel.

Check significance of the effects. The significance was assessed using Analysis of the Variance (ANOVA) and using P-value significance levels. This value represents the probability of the effect of a factor being due solely to random error. Thus, if the P value is less than 5%, the effect of the corresponding factor is significant.

Doehlert design and Lagrange criterium. We chose the Doehlert matrix design in this case because it is a uniform shell design where the factor co-ordinates of experimental points are equally spaced about centred circles, spheres or hyperspheres (a hexagon in the case of two variables). The whole experimental domain is explored with a minimum number of experiments following a sequential approach. K factors require k²+k+1 experiments and a minimum of three levels is assigned to each factor. With the two-factor design, the Doehlert matrix dimensions are [5,3]. This property allows the free choice of the factors to be assigned to a large or small number of levels.

The experimental domain is defined by its centre and by the variation of each factor around the centre. The theoretical Doehlert matrix leads to a theoretical experimental design set with coded values (C). The relationship between coded and real values is given by

\[ C_i = (X_i - X_0^*)/(CX_i)K \]

where \( X_0^* \) is the real value at the centre of the experimental domain, \( CX_i \), the step of variation of the real value, and K is equal to 1 and 0.866 for the first and second factor, respectively.

From the experimental responses, polynomial functions, \( Y = f(A,B) \) can be expressed as:

\[ Y = a_0 + a_1(A) + a_2(B) + a_3(A^2) + a_4(B^2) + a_5(AB) \]

The coefficients (aₙ), which constitute the model parameters,

are calculated by using a least-squares multiple regression method: $a_0$ is the independent term; $a_1$ and $a_2$ are the coefficients of the linear terms; $a_3$ and $a_4$ are the coefficients of the quadratic terms; and $as$ is the coefficient of the interaction term. The model accuracy is given by the $R$-squared statistics ($R^2$). Coefficients with confidence levels below 90% are not considered.

The objective of Lagrange’s criterion is to locate the critical point of the $2^nd$ order equation and it is based on calculating the Hessian determinant of $Y$.

$$H(A,B) = (\delta^2 Y / \delta A^2)(\delta^2 Y / \delta B^2) - (\delta^2 Y / \delta A \delta B)$$

The critical point $(a_0,b_0)$ is maximum if $H(a_0,b_0) > 0$ and $\delta^2 Y / \delta A^2 < 0$, it is minimum if $H(a_0,b_0) < 0$ and $\delta^2 Y / \delta A^2 > 0$, or a saddle point exists, if $H(a_0,b_0) = 0$. If the response surface has a maximum, the co-ordinate is calculated by solving the system of equations: $\delta Y / \delta A = 0$ and $\delta Y / \delta B = 0$.

The arrangement of a new design depends, above all, on the results derived from applying the Lagrange criterion to the function of the estimated response. The lack of a maximum, within the experimental zone enclosed in the design, would require a new design to be arranged in the region where the desired optimum is thought to be.

### Results and discussion

#### Optimization of the preconcentration procedure

To optimize the system, efforts were focused on the conditions for sample loading and metal elution from the column, as well as the analytical flow system which was coupled on-line with the preconcentration and separation unit in order to obtain highly sensitive, accurate and reproducible results.

To minimize the time needed for quantitative elution and to facilitate eluate delivery, dispersion during elution and eluate introduction must be reduced, while a highly efficient eluent should be used. A mixture of citric acid and tartaric acid was chosen as the eluent owing to its effective elution of the adsorbed analyte complex, its easy direct delivery through the peristaltic pump tubing and its low toxicity.

Preliminary tests showed that the sample volume was not an important factor when the mass of analyte arriving at the column was kept constant. On the other hand, the effect of sample loading time on the emission signal of $100$ ng ml$^{-1}$ Zn was studied at a sample flow rate of 6 ml min$^{-1}$ using a microcolumn (3 cm x 3 mm id) packed with the chelating resin to a height of 0.7 and 1.0 cm. The signal increased almost linearly up to a 12 and 15 min preconcentration time, respectively, after which the slope decreased gradually, presumably as a result of insufficient capacity of the resin. This was confirmed using a longer column. However, longer columns are more susceptible to back-pressure problems, owing to packing of the resin beads, and therefore need more frequent replacement. Hence, a glass tube microcolumn (3 cm x 3 mm id), packed with the DPTH-gel resin to a height of 0.7 cm, was selected for this study. Sensitivity enhancements increased by increasing the sample loading time; however, a loading time of 60 s was selected in order to achieve high sampling frequency with a reasonable degree of sensitivity. A longer loading time can be employed for samples with low concentrations of metal.

Once these factors had been fixed, the variables to be optimized were: sample flow rate, eluent flow rate, eluent concentration, pH and buffer concentration. In the optimization process the mass of analyte arriving at the column was fixed at 300 ng in all instances.

#### Factorial design

A two-level full factorial design, $2^4$, involving 32 runs was used as a first approximation to the response surface of the preconcentration process. Table 3 shows the experimental design matrix and the results obtained in each run expressed as average peak height. The results of the variance analysis (ANOVA) carried out on the data given in Table 3 are shown in Table 4.

It can be deduced that the four factors, eluent flow rate ($P_1$), buffer concentration ($B$), sample flow rate ($P_2$) and pH ($\text{pH}$), and also the interactions ($P_2B$ and $B\cdot\text{pH}$), are significant. It is important to note that the eluent concentration and its interaction with the other factors are not significant. From this first experiment, one deduces the necessity of continuing the optimization, because the effects are highly significant on four variables ($P_1$, $B$, $P_2$, and $\text{pH}$), thus indicating that the response surface varied greatly. The eluent concentration was fixed at 1% citric acid +1% tartaric acid in the succeeding experiments.

#### Doehlert design

In this optimization strategy two designs of two factors are performed, giving two response surfaces, one for each pair of factors. Thus, by using the design corresponding to the second pair in the optimum conditions obtained from the first, the optimum conditions are found for the experimental domain assayed.
Design (a). In this design, the variables to be optimized were the eluent flow rate and the buffer concentration, maintaining sample flow rate and the pH constant at 6 ml min\(^{-1}\) and 6, respectively. The seven experiments required for the Doehlert matrix and the experimental results obtained for the net emission signal are grouped in Table 5; three levels were assigned to one variable, the eluent flow rate, and five to the other, the buffer concentration.

The results obtained (as real values, not codified) fit the equation:

\[ Y = -122.677 + 52.6759P_1 + 209.16B - 4.53966P_1^2 - 23475.5B^2 + 108.184P_1B \]

where \( Y \) is the net emission signal and \( P_1 \) and \( B \) are sample flow rate in ml min\(^{-1}\) and buffer concentration in moles per litre, respectively. The predicted values by this equation are in accordance with the peak height values obtained experimentally (coefficient of determination, \( R^2 = 0.998 \)).

The response surface for this equation is shown in Fig. 4(a). The application of Lagrange’s criterion to the latter equation indicates the presence of a maximum located at the position given by 6.5 ml min\(^{-1}\) \( P_1 \) and 0.06 mol l\(^{-1}\) \( B \), which were chosen for latter experiments. As can be seen from the Fig. 4(a), these values fall within the experimental area.

Design (b). In this instance, eluent flow rate and buffer concentration were kept constant at the optimum values found previously and the sample flow rate and pH were optimized. The seven experiments required for the Doehlert matrix and the experimental results obtained for the emission signal are given in Table 5.

The results obtained fit the equation:

\[ Y = -706.004 - 0.8266P_1 + 168.529pH - 1.43458P_1^2 - 9.60375pH^2 + 2.6133P_1pH \]

After verifying that the second order model fits the experimental data \( (R^2 = 0.998) \), the application of Lagrange’s criterion indicates the presence of a maximum located at the position 8.8 ml min\(^{-1}\) \( P_1 \) and 9.9 in pH, which, it can be seen, fall within the experimental domain.

As a result of these observations, the following working conditions were chosen: \( P_1 = 6.5 \) ml min\(^{-1}\), \( B = 0.06 \) mol l\(^{-1}\), \( P_2 = 8.8 \) ml min\(^{-1}\), \( pH = 9.9 \) and \( B \) was fixed at 1% citric acid + 1% tartaric acid. Under these conditions, the peak intensity was approximately 3.5 times greater than the lowest peak intensity obtained with the first design, and the analytical quality of the signal was guaranteed.

Performance of the method

Under the optimum conditions, with the use of a 60 s preconcentration time, a sample flow rate of 8.8 ml min\(^{-1}\) and an elution flow rate of 6.5 ml min\(^{-1}\), a linear calibration graph was obtained from 2 to at least 500 ng ml\(^{-1}\) of Zn(II) with a regression coefficient of 0.9994. The signal appeared 20 s after sample injection. Within 40 s after injection, the FI system was ready for the next injection, thus giving a throughput of about 40 samples h\(^{-1}\).

The detection limit, defined as the concentration of analyte giving a signal equivalent to three times the standard deviation of the blank signal plus the net blank intensity, was measured to be 1.7 ng ml\(^{-1}\); the absolute blank value was obtained by using the preconcentration and determination procedures with de-ionized water buffered at pH 9.9.

The results of including significant levels of possible interferents are presented in Table 6. For this study, different amounts of the ionic species tested were added to a 100 ng ml\(^{-1}\) solution of zinc. The starting point was an interference: zinc ratio of 2000 m/m; if any interference occurred, the ratio was gradually lowered until the interference disappeared. The preconcentration of zinc was reduced in the presence of relatively high concentrations (at a level 500-fold in excess of zinc) of Mn(II), Ni(II), Co(II) and Cd(II) because these elements can be chelated by the column and compete with zinc for the active sites.

The precision of the method for aqueous standards (evaluated as the relative standard deviation obtained after analysing ten series of ten replicates) was 0.8% at the 100 ng ml\(^{-1}\) level of Zn(II) and 1.5% at the 10 ng ml\(^{-1}\) level.

In order to test the accuracy and applicability of the proposed method to the analysis of real samples, two reference materials were analysed. The results, as the average of three separate determinations, are shown in Table 7. The results showed that the proposed automated system provides analytical data within the ranges of the certified values.

In view of the application of the method to the determination of zinc in water, standard solutions containing Zn(II) were added to synthetic sea water. The results, shown in Table 7, indicated excellent recovery.

Conclusions

Optimization of a FI preconcentration and matrix separation on solid sorbents procedure, based on a traditional univariate approach, involves a great many experiments; the more variables the more experiments. In this study it has been

Table 4: Analysis of data given in Table 3

<table>
<thead>
<tr>
<th>Source</th>
<th>SS*</th>
<th>DF*</th>
<th>MS*</th>
<th>F-ratio</th>
<th>P level**</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P_1 )</td>
<td>270.281</td>
<td>1</td>
<td>270.281</td>
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<td>( P_2 )</td>
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<td>( E )</td>
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<td>26.2813</td>
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<tr>
<td>( B )</td>
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<td>4072.53</td>
<td>149.28</td>
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</tr>
<tr>
<td>( pH )</td>
<td>1069.53</td>
<td>1</td>
<td>1069.53</td>
<td>39.20</td>
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<tr>
<td>( P_1 \cdot P_2 )</td>
<td>1.53125</td>
<td>1</td>
<td>1.53125</td>
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<tr>
<td>( P_1 \cdot E )</td>
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<td>258.781</td>
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<td>0.0072</td>
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<tr>
<td>( P_1 \cdot B )</td>
<td>22.7813</td>
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<td>22.7813</td>
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Table 5: (a) Optimization of \( P_1 \) and \( B \); (b) Optimization of \( P_2 \) and pH

<table>
<thead>
<tr>
<th>Design (a)</th>
<th>Experiment</th>
<th>( P_1^a )</th>
<th>( B^b )</th>
<th>( H^c )</th>
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<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>0.0325</td>
<td>43.8</td>
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<tr>
<td>2</td>
<td>9</td>
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<table>
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<th>Design (b)</th>
<th>Experiment</th>
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<th>( pH^b )</th>
<th>( H^c )</th>
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<td>14</td>
<td>3</td>
<td>10</td>
<td>82.98</td>
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</tr>
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</table>

*\( P_1 \), eluent flow rate (ml min\(^{-1}\)); \( B \), buffer concentration (mol l\(^{-1}\)).
*\( H \), peak height (mV); \( P_2 \), sample flow rate (ml min\(^{-1}\)).
shown that the application of a Doehlert design considerably reduces the amount of experimental work required. By using such a design we have been able to establish the optimum conditions for the preconcentration of Zn(II). Furthermore, as such a design we have been able to establish the optimum conditions for the preconcentration of Zn(II). Furthermore, as such a design we have been able to establish the optimum conditions for the preconcentration of Zn(II). Furthermore, as such a design we have been able to establish the optimum conditions for the preconcentration of Zn(II). Furthermore, as such a design we have been able to establish the optimum conditions for the preconcentration of Zn(II). Furthermore, as such a design we have been able to establish the optimum conditions for the preconcentration of Zn(II). Furthermore, as such a design we have been able to establish the optimum conditions for the preconcentration of Zn(II). 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