Application of uniform design and genetic algorithm in optimization of reversed-phase chromatographic separation

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Abstract

An optimization method based on uniform design in conjunction with genetic algorithm is described. According to the proposed method, the uniform design technique was applied to the design of starting experiments, which can reduce the number of experiments compared with traditional simultaneous methods, such as simplex. And genetic algorithm was used in optimization procedure, which can improve the rapidity of optimal procedure. The hierarchical chromatographic response function was modified to evaluate the separation equality of a chromatogram. An iterative procedure was adopted to search for the optimal condition to improve the accuracy of predicted retention and the quality of the chromatogram. The optimization procedure was tested in optimization of the chromatographic separation of 11 alkaloids in reversed-phase ion pair chromatography and satisfactory optimal result was obtained.

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1. Introduction

Optimization of chromatographic separation is always an active research area because it is one of the key subjects for intelligent and automatic chromatographic separations. Many methods have been developed in order to optimize the parameters of interest in chromatography [1–7], which can be divided into two categories [1]. One is the sequential method, in which experiments are performed without consideration of the solute retention before the design of the experiments, such as simplex [2], complex [3], and iterative design [4]. The optimization results obtained with these methods have high accuracy, but the optimum is not global and many experiments are required. The other is the simultaneous method, where experiments are performed after the design of the starting experiment, such as window diagrams [5], overlapping resolution mapping [6], and critical bands [7]. With these methods, the global optimum can be achieved with less experiments, but the accuracy of the optimization result is not as high as that obtained with the sequential methods.

Genetic algorithm is an optimization algorithm that mimics the mechanisms of natural selection described by genetics and the Darwinian theory of evolution. As a global-searching algorithm, genetic algorithm makes use of artificial intelligence to obtain the solution of rather complex problems, so it has been widely applied.
in resolving combinatorial optimization problems [8], and attempter problems [9] due to their parallelism and effective utilization of global information. However, up to now, there are a few reports on the application of genetic algorithm in optimization of chromatography [10–12]. Genetic algorithm has been applied in source identification of underground fuel spills by Lavine et al. [10] and in prediction for chromatographic retention by Zhang et al. [11]. Recently, Nikitas et al. [12] used genetic algorithm in response surface modeling in high-performance liquid chromatography. Since genetic algorithm can offer a good performance in both solution quality and algorithm speed in parameters optimization, and the optimization of mobile phase composition in high-performance liquid chromatography is a typical process for parameter optimization, then it is necessary to explore the application of genetic algorithm to the optimization of mobile phase composition in liquid chromatography. In this paper, an optimization method by combining uniform design with genetic algorithm [13] has been developed for the optimization of mobile phase composition in reversed-phase ion pair liquid chromatography.

2. Optimization strategy

The flow chart for the optimization procedures is shown in Fig. 1, which can be expressed as following.

2.1. Selection of an optimal column system

The right column system is necessary for the optimization of chromatographic separations. Therefore, an optimal column system that includes separation mode and mobile phase constituents and stationary phases, etc., should be selected at first. In this work, 11 alkaloids were selected to test the optimization method. The separation of alkaloids can be performed in reversed phase liquid chromatography [1,14–16]. It was suggested that methanol is the most widely used organic modifier [16], and addition of ion pair reagent such as D-camphor-10-sulfonic acid in the mobile phase can improve the selectivity and resolution, thus reversed-phase ion pair liquid chromatography using methanol as the organic modifier and D-camphor-10-sulfonic acid as the ion pair reagent was recommended for the separation of the tested alkaloids.

2.2. Screening for optimization parameters

According to physical and chemical characters of the samples, the factors that affect separation markedly can be selected as the optimization parameters. For the selected optimal column system, reversed-phase ion pair liquid chromatography, the concentrations of organic modifier and ion pair reagent play the key roles in the retention behavior of solutes. Therefore, the volume fraction of methanol and the concentration of D-camphor-10-sulfonic acid were chosen as optimization parameters.

2.3. Definition of optimal region

When optimization parameters have been selected, defining their region can ensure that experiments within the region are practicable. In this work, according to Ref. [16], the optimization region selected was 20% to 50% (% vol) for methanol and 1.00 to 15.00 mmol/l for D-camphor-10-sulfonic acid.

2.4. Design of starting experiments

In order to test the rule of the optimal parameters in the range of optimization, an equation that accurately describes the effects of variables on the
retention of solutes is necessary. Therefore, setting the starting experiments to represent the whole range of factors by statistical methods is required, and the rule of the retention of solutes in the range of optimization can be expressed with these typical experiments. A kind of technique for experiment design, named the uniform design [17–19], can be chosen to design the starting experiments with the purpose of these. As a design method, the uniform design method not only considers the regularity but also the uniformity [17–19], so it is an appropriate alternative.

2.5. Performance of the starting experiments

These starting experiments with various conditions were accomplished; the retention of all solutes and the peak widths at half height of completely resolved peaks were measured.

2.6. Fitting of retention equation

In our past work [1,20], the chromatographic retention model was built based on the chromatographic theory, which can describe the rule between the retention factor and mobile phase composition. In reversed-phase ion pair chromatography, the retention equation of each component could be expressed as follows [20]:

\[
\ln k = a + b \ln C_M + c C_M + d \ln C_T + e C_T + f C_M C_T
\]  

(1)

where \( k \) is retention factor, \( a, b, c, d, e \) and \( f \) are coefficients, \( C_M \) and \( C_T \) are the concentrations of methanol and \( \delta \)-camphor-10-sulfonic acid, respectively. The coefficients in Eq. (1) could be obtained by regression analysis of initial data from the starting experiments. The linear relationship between the peak width at half height (\( W_{1/2} \)) and the retention time (\( t_R \)) of solutes was also performed with linear regression analysis as follows:

\[
W_{1/2} = a_w + b_w \times t_R
\]  

(2)

where \( a_w \) and \( b_w \) are coefficients. Then the retention time of the solutes and the peak width at half height for the peaks at various conditions could be predicted with these two equations, respectively.

2.7. Searching for the optimization point

Genetic algorithm based on line-crossover and plane-mutation was applied to search the optimization separation condition. The details will be discussed in the following section.

2.8. Iterative optimization

Once the optimization separation condition is obtained, the experiment can be accomplished under this condition. Then the experimental result is judged whether one of the end requirements is satisfied or not. If it does, the procedure is stopped, otherwise, steps from 2.6 to 2.7 are iterated. The experimental data presently obtained will be added into the previous data set, the retention equations will be modified, the optimization point will be searched again. Then a new point can be obtained. The requirements to end optimization include: (1) The hierarchical chromatography response function (HCRF) [21] value obtained from the data under the previous separation condition is same or smaller than the last maximum value of HCRF; (2) the separation condition currently obtained is same as the previous; (3) the separation results obtained are reasonable for analysis of the samples. Once one of the requirements is fulfilled, the whole optimization procedures are ended.

3. Principle for application of genetic algorithm in optimization of separation condition

The principle for application of genetic algorithm in optimization of chromatographic separation is shown in Fig. 2.

In our case, two parameters, the concentrations of methanol and \( \delta \)-camphor-10-sulfonic acid, were chosen to be optimized. Thus, two-dimensional points were selected to denote the individuals and the coordinates of these points denote the concentrations of methanol and \( \delta \)-camphor-10-sulfonic acid in our system, and the concentrations were encoded in real number. The procedures in details can be expressed as follows.
3.1. Initialization

Genetic algorithm does not operate on an individual point for searching the parameter space but on a group of points (called population) at a time. Generally, an initial group includes 20 to 300 points. In this work, an initial group containing 30 two-dimensional points was generated with the uniform design method [17–19] in the region of optimization parameters.

3.2. Line-crossover

Line-crossover was the major operation of genetic algorithm, the detail is illustrated in Fig. 3. It is supposed that A and B are the cross points. Drawing a line between A and B, setting the midpoint of AB as C, then extending BA to D, where A is the midpoint of CD, then extending AB to E, just fitting B is the midpoint of CE. After these operations, three points, which are offspring of A and B, are obtained.

3.3. Plane-mutation

The major operation of plane-mutation is subtracting and adding of variables. It is supposed that $H(C_M,C_T)$ is the point before mutation, and $J(C_M,C_T)$ is the point after mutation, thus $J = H + A$, where $A$ is mutation range ($A = \{a_1, a_2\}$). In addition, mutation range should be reduced after one mutation, $A'$ is defined as the new mutation range after mutation, thus $A' = \alpha \times A$, where $\alpha$ is the reduced coefficient. The value of the reduced coefficient is 0.99 in general. In this work, every individual is a two-dimensional point, the operation of plane-mutation was illustrated in Fig. 4 in detail. It is supposed that O is the plane-mutation point, after the operation, eight points, which are offspring of O, are obtained.

3.4. Arranging with the fitting values

The retention time and peak widths at half height of all solutes are firstly predicted according to Eqs. (1) and (2) with $C_M$ and $C_T$ denoted by the points (offspring) to be arranged. Then the resolution ($R_s$) [1] of neighbor pair peaks can be counted. If $R_s > 1.0$, it means that two solutes can be separated according to the $R_s$ value. Otherwise, two solutes cannot be resolved if $R_s < 1.0$. A hierarchical chromatographic response function (HCRF) [21] is modified and used to evaluate the quality of a chromatogram as follows:

$$
HCRF = 1,000,000 \times n + 100,000 \times R_{\text{min}} + (T_D - T_L)$$

(3)

where $n$ is the number of resolved peaks in the chromatogram. $R_{\text{min}}$ is the resolution for the least-resolved pair of peaks (when $R_{\text{min}} > 1.60$, which means that all components can be baseline-separated, then $R_{\text{min}}$ is specified as 1.60). $T_D$ and $T_L$ are the maximum acceptable analysis time and the retention time of the last peak, respectively. If $T_L$ is larger than $T_D$, it means that the analysis time is too long to be accepted, and then HCRF is specified as 1,000,000.00. Finally, all the points are arranged according to their HCRF value.

3.5. Replacement of worse parents by better children

Certain numbers of better children are chosen to replace certain numbers of worse parents, which can
keep parents in certain number and make parents evolved. In this work, five better children were chosen to replace five worse parents.

According to the stop criterion of genetic algorithm calculation, it will be stopped if the best individual is not replaced for 20 times.

4. Experimental

4.1. Apparatus

All experiments were performed on the HPLC instrument combining a Waters HPLC 515 pump with 2487 dual \( \lambda \) absorbance detector (Milford, MA, USA) and WDL-95 workstation (National Chromatographic R&A Center, China).

Separations were carried out on a 150 × 4.6-mm I.D. column packed with 5 \( \mu \)m Kromasil-C\(_{18}\) (National Chromatographic R&A Center), flow rate is set at 1.0 ml/min and detection wavelength at 254 nm.

4.2. Reagents

Cinchonine, theobromine, nicotine, theophylline, atenolol, caffeine, barbital, gramine, phenylbarbita, metoprolol and sulfanilamide were purchased from Sigma (St. Louis, MO, USA); methanol and monosodium phosphate were purchased from Shenyang Regent Factory (Shenyang, China); \( \alpha \)-camphor-10-sulfonic acid was purchased from East China Normal University (Shanghai, China); distilled water used in all experiments was purified by a Milli Q system (Milford, MA, USA).

5. Results and discussion

According to physical and chemical characters of samples, reversed-phase ion pair liquid chromatography with ODS column has been chosen as the separation mode for 11 alkaloids solutes. Mobile phase was composed of methanol-phosphate buffer (50 mmol/l) at pH 4.50 by addition of ion pair reagent of \( \alpha \)-camphor-10-sulfonic acid. The volume fraction of methanol and the concentration of \( \alpha \)-camphor-10-sulfonic acid were the optimization variables ranging from 20% to 50% (\%, v/v) and from 1.00 to 15.00 mmol/l, respectively.

According to the uniform design method\[17–19\], from table \( U_7 \) (7\(^6\)) and its application table, seven starting experiments were designed as shown in Fig. 5. The concentrations of methanol and \( \alpha \)-camphor-10-sulfonic acid for these seven starting experiments were distributed in the variable region uniformly. The values of \( k \) of individual solutes and \( W_{1/2} \) for resolved peaks were measured under the conditions for starting experiments. The regression analyses between the logarithms of \( k \) and concentration of methanol and \( \alpha \)-camphor-10-sulfonic acid as well as that between \( W_{1/2} \) and \( t_R \) were carried out according to Eqs. (1) and (2), respectively. Coefficients in Eq. (1) and the correlation coefficient calculated from the initial experimental data for 11 alkaloids are listed in Table 1. Genetic algorithm based on line-crossover and plane-
mutation was accomplished as follows. (1) An initial group containing 30 two-dimensional points was generated with the uniform design method [17–19] in the region of optimization parameters. (2) These points were divided into 15 units, line-crossover was operated on each unit, and the number of offspring generation was 45 when these operations were accomplished. (3) Following line-crossover, plane-mutation was operated on the 45 offspring, and then 360 points were generated, which were specified as the offspring of the initial 30 individuals. (4) The retention time and peak widths at half height of all solutes were predicted according to Eqs. (1) and (2) with $C_M$ and $C_T$ denoted by these 360 points, then these 360 points were arranged according their corresponding HCRF values calculated by Eq. (3). (5) Then five better points with maximum HCRF values were selected to replace five worse points with minimum HCRF values in the initial group. And the best point with the maximum HCRF value was selected as the best offspring in this cycle, and the maximum HCRF value previously obtained should be replaced by that value currently obtained if it improved. Then if stop criterion was not agreed, steps from (2) to (5) were iterated until the stop criterion was agreed. Thus, the optimum point was obtained with the volume fraction of methanol at 29% (%, v/v) and the concentration of D-camphor-10-sulfonic acid at 9.00 mmol/l, respectively, which was specified as the composition for the 8th experiment. Separation of 11 alkaloids was performed under the specified condition, and the obtained chromatogram is shown in Fig. 6. It can be seen in Fig. 6 that the HCRF value is $11,153,542.43$, and the solutes with Nos. 4 and 5 were not completely resolved under this separation condition.

Because none of the requirements was satisfied to end the optimization procedure, the retention values of the solutes measured at the 8th experiment were taken into the regression analysis according to Eq. (1). Modified coefficients in retention Eq. (1) and the correlated coefficient after introduction of optimizing point for 11 alkaloids are listed in Table 2. Then the parameters were optimized accordingly and a new separation condition could be found with methanol content of 27% (%, v/v) and D-camphor-10-sulfonic acid content of 10.00 mmol/l, which was specified as the composition for the 9th experiment. The separation of 11 alkaloids was performed again under the new condition, and the obtained chromatogram is shown in Fig. 7.

It can be seen that the HCRF value at the 9th experiment is $11,160,900.17$, which is larger than that

<table>
<thead>
<tr>
<th>Solute</th>
<th>$a$</th>
<th>$b$</th>
<th>$c$</th>
<th>$d$</th>
<th>$e$</th>
<th>$f$</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
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<td>Cinchonine</td>
<td>539.06</td>
<td>203.34</td>
<td>-808.09</td>
<td>-46.02</td>
<td>2.39</td>
<td>9.97</td>
<td>0.9602</td>
</tr>
<tr>
<td>Theobromine</td>
<td>390.54</td>
<td>149.08</td>
<td>-629.22</td>
<td>-20.57</td>
<td>-0.63</td>
<td>10.54</td>
<td>0.9967</td>
</tr>
<tr>
<td>Nicotine</td>
<td>240.99</td>
<td>99.12</td>
<td>-450.07</td>
<td>8.17</td>
<td>-3.53</td>
<td>10.15</td>
<td>0.9967</td>
</tr>
<tr>
<td>Theophylline</td>
<td>253.54</td>
<td>94.38</td>
<td>-399.68</td>
<td>-16.79</td>
<td>0.04</td>
<td>6.4</td>
<td>0.9979</td>
</tr>
<tr>
<td>Atenolol</td>
<td>235.05</td>
<td>94.37</td>
<td>-421.7</td>
<td>2.22</td>
<td>-2.53</td>
<td>8.82</td>
<td>0.9976</td>
</tr>
<tr>
<td>Caffeine</td>
<td>288.81</td>
<td>119.9</td>
<td>-534.82</td>
<td>10.38</td>
<td>-4.17</td>
<td>11.83</td>
<td>0.9966</td>
</tr>
<tr>
<td>Barbital</td>
<td>326.08</td>
<td>129.09</td>
<td>-558.75</td>
<td>-4.06</td>
<td>-2.4</td>
<td>10.75</td>
<td>0.9979</td>
</tr>
<tr>
<td>Gramine</td>
<td>379.82</td>
<td>161.42</td>
<td>-717.65</td>
<td>20.39</td>
<td>-6.26</td>
<td>16.12</td>
<td>0.9998</td>
</tr>
<tr>
<td>Phenylbarbital</td>
<td>353.97</td>
<td>141.26</td>
<td>-614.01</td>
<td>-0.67</td>
<td>-3.1</td>
<td>12.05</td>
<td>0.9995</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>284.41</td>
<td>105.37</td>
<td>-450.74</td>
<td>-16.87</td>
<td>-0.14</td>
<td>7.21</td>
<td>0.9996</td>
</tr>
<tr>
<td>Sulfanilamide</td>
<td>469.92</td>
<td>183.56</td>
<td>-763.05</td>
<td>-17.59</td>
<td>-1.13</td>
<td>12.09</td>
<td>0.9984</td>
</tr>
</tbody>
</table>
at the 8th experiment, thus none of the requirements is satisfied to optimization procedure, and the optimization calculation will be carried out again. Another optimum was obtained with the concentration of methanol at 27% (%, v/v) and D-camphor-10-sulfonic acid content at 10.00 mmol/l, respectively, which was specified for the 10th experiment. It can be seen that the obtained concentration of methanol and D-camphor-10-sulfonic acid was the same as that obtained for the 9th experiment. Thus, one of the requirements to end the optimization procedure was fulfilled, and the optimization procedure could be stopped and the concentration of methanol and D-camphor-10-sulfonic acid for the 10th experiment was regarded as the final optimum.

Table 3 lists the concentrations of methanol and D-camphor-10-sulfonic acid in various experiments and their corresponding HCRF values for the chromatograms obtained experimentally. In Table 3, the sequence numbers from 1 to 7 are the starting experiments designed by the uniform design method, the sequence numbers from 8 to 10 are the experi-

<table>
<thead>
<tr>
<th>Solute</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinchonine</td>
<td>537.74</td>
<td>202.82</td>
<td>−806.44</td>
<td>−45.9</td>
<td>2.39</td>
<td>9.95</td>
<td>0.9607</td>
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<td>Theobromine</td>
<td>388.8</td>
<td>148.41</td>
<td>−627.06</td>
<td>−20.41</td>
<td>−0.63</td>
<td>10.51</td>
<td>0.9968</td>
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<tr>
<td>Nicotine</td>
<td>242.27</td>
<td>99.62</td>
<td>−451.66</td>
<td>8.05</td>
<td>−3.53</td>
<td>10.17</td>
<td>0.9966</td>
</tr>
<tr>
<td>Theophylline</td>
<td>252.2</td>
<td>93.86</td>
<td>−398.01</td>
<td>−16.67</td>
<td>0.04</td>
<td>6.38</td>
<td>0.9979</td>
</tr>
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<td>Atenolol</td>
<td>236.19</td>
<td>94.82</td>
<td>−423.12</td>
<td>2.12</td>
<td>−2.53</td>
<td>8.84</td>
<td>0.9976</td>
</tr>
<tr>
<td>Caffeine</td>
<td>287.66</td>
<td>119.45</td>
<td>−533.39</td>
<td>10.48</td>
<td>−4.17</td>
<td>11.81</td>
<td>0.9966</td>
</tr>
<tr>
<td>Barbitol</td>
<td>325.02</td>
<td>128.67</td>
<td>−557.42</td>
<td>−3.96</td>
<td>−2.4</td>
<td>10.74</td>
<td>0.998</td>
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<tr>
<td>Gramine</td>
<td>379.02</td>
<td>161.11</td>
<td>−716.65</td>
<td>20.46</td>
<td>−6.26</td>
<td>16.1</td>
<td>0.9998</td>
</tr>
<tr>
<td>Phenylbarbitol</td>
<td>354.23</td>
<td>141.36</td>
<td>−614.34</td>
<td>−0.69</td>
<td>−3.09</td>
<td>12.06</td>
<td>0.9995</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>283.58</td>
<td>105.05</td>
<td>−449.7</td>
<td>−16.8</td>
<td>−0.14</td>
<td>7.19</td>
<td>0.9996</td>
</tr>
<tr>
<td>Sulfanilamide</td>
<td>469.72</td>
<td>183.49</td>
<td>−762.8</td>
<td>−17.57</td>
<td>−1.13</td>
<td>12.08</td>
<td>0.9984</td>
</tr>
</tbody>
</table>

Fig. 6. Chromatogram at the first optimum point. Experimental conditions: mobile phase, methanol–50 mmol/l phosphate buffer (29/71, v/v, pH 4.50) containing 9.00 mmol/l D-camphor-10-sulfonic acid; column, 150 × 4.6 mm I.D. packed with 5 μm Kromasil ODS; flow rate, 1.0 ml/min; detection wavelength, 254 nm. Solutes: (1) cinchonine; (2) theobromine; (3) nicotine; (4) theophylline; (5) atenolol; (6) caffeine; (7) barbital; (8) gramine; (9) phenylbarbital; (10) metoprolol; (11) sulfanilamide.
ments under the optimal conditions using genetic algorithm. Table 4 lists the retention times experimentally measured and predicted for the 11 alkaloids with sequences numbers 8 and 9, respectively. It can be seen that the HCRF value at the 9th experiment is much higher than that at the 8th experiment. Both the maximum deviation and the average deviation of retention time decrease quickly, which means that the accuracy to search for the optimal condition is enhanced greatly, and the prediction condition is near to the final optimal condition.

Based on uniform design and genetic algorithm, the optimization method has two characteristics. One is uniform design, which makes it possible for the starting experiments to be uniformly located and the retention of solutes to be more accurately predicted in the range of variables to be optimized. Therefore, the disadvantages of the sequential approaches such as simplex for the local optimum can be overcome. The

### Table 3
Sequence number for experiments and the corresponding concentrations of methanol and α-camphor-10-sulfonic acid as well as the value of HCRF

<table>
<thead>
<tr>
<th>Sequence number for experiments</th>
<th>C_M</th>
<th>C_T</th>
<th>HCRF</th>
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<tr>
<td>Starting experiments</td>
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<tr>
<td>1</td>
<td>20</td>
<td>5.66</td>
<td>11,160,721.96</td>
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<tr>
<td>2</td>
<td>25</td>
<td>12.66</td>
<td>11,160,806.72</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>3.33</td>
<td>10,102,370.55</td>
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<tr>
<td>4</td>
<td>35</td>
<td>10.33</td>
<td>9,019,480.49</td>
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<tr>
<td>5</td>
<td>40</td>
<td>1.00</td>
<td>9,039,994.42</td>
</tr>
<tr>
<td>6</td>
<td>45</td>
<td>8.00</td>
<td>8,052,892.22</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>15.00</td>
<td>8,036,515.58</td>
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<tr>
<td>Optimized experiments</td>
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<tr>
<td>8</td>
<td>29</td>
<td>9.00</td>
<td>11,153,542.43</td>
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<tr>
<td>9</td>
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<tr>
<td>10</td>
<td>27</td>
<td>10.00</td>
<td>11,160,900.17</td>
</tr>
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### Table 4
Predicted (t_p) and experimental (t_e) retention time of solutes at the optimums and relative deviation (RD) between them

<table>
<thead>
<tr>
<th>Solute</th>
<th>No.</th>
<th>First optimum</th>
<th>Second optimum</th>
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<tbody>
<tr>
<td></td>
<td>t_p</td>
<td>t_e</td>
<td>RD (%)</td>
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<td>1.87</td>
<td>1.88</td>
</tr>
<tr>
<td>Theobromine</td>
<td>2</td>
<td>2.65</td>
<td>2.7</td>
</tr>
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<td>3</td>
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</tr>
<tr>
<td>Theophylline</td>
<td>4</td>
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<td>4.4</td>
</tr>
<tr>
<td>Atenolol</td>
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<td>4.67</td>
<td>4.68</td>
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<tr>
<td>Caffeine</td>
<td>6</td>
<td>6.35</td>
<td>6.32</td>
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<td>Barbital</td>
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<td>8.4</td>
<td>8.5</td>
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<tr>
<td>Gramine</td>
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<td>14.25</td>
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<tr>
<td>Phenylbarbital</td>
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<td>30.88</td>
<td>30.51</td>
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<tr>
<td>Sulfanilamide</td>
<td>11</td>
<td>76.24</td>
<td>78.22</td>
</tr>
</tbody>
</table>

RD_{max} 2.53 1.06
RD_{ave} 1.30 0.78

Fig. 7. Chromatogram at the second optimum. Experimental conditions: mobile phase, methanol– 50 mmol/l phosphate buffer (27/73, v/v, pH 4.5) containing 10.00 mmol/l α-camphor-10-sulfonic acid. Other conditions and solutes are the same as in Fig. 6.
other is genetic algorithm, which makes it possible to increase the accuracy for prediction of the retention times of solutes. Moreover, the global optimum can be obtained.

An iterative optimization method with step-length searching and iterative optimization previously developed [22] was also used in optimizing the separation condition of alkaloids. Table 5 shows the results with two optimization methods. It can be seen that both methods give the same optimum results. However, much less time is needed by uniform design and genetic algorithm, compared to the previous method. That is because the optimizing procedure in the previous method is performed on the whole range of parameters, and depends on the accuracy of seeking, in other words, the accuracy is higher, and the time consumed is longer. Whereas, genetic algorithm is operated in the range of optimization region with evolution rules and there is no relation between the time consumed and the accuracy of seeking. Therefore, the optimization method using uniform design and genetic algorithm is more efficient than the previous method.

6. Conclusion

The optimization method using uniform design and genetic algorithm was applied in optimizing the chromatographic separation in reversed-phase ion pair chromatography mode. The process in optimizing the content of methanol and D-camphor-10-sulfonic acid (ion pair reagent) in the mobile phase by this optimization method was introduced in detail. Eleven alkaloids were selected to test the optimization method and the optimum result was obtained by three times of optimization calculation and the average relative deviation between predicted and experimental values was only 0.78% under the optimum separation condition. Due to the advantages of the genetic algorithm, the optimization method using uniform design and genetic algorithm has potential applications not only for the two-parameter optimization but also for multi-parameters optimization.

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References


