

# Prediction of Retention Times, Column Efficiency, and Resolution in Isothermal and Temperature-Programmed Gas Chromatography: Application for Separation of Four Psoralens

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## Abstract

A chemometric methodology is proposed to study the retention time, height equivalent to a theoretical plate, and separation of four psoralens in both isothermal and temperature-programmed gas chromatography. In temperature-programmed conditions, 18 experiments are found to be necessary, instead of nine in isothermal conditions. The differences between the predicted and the experimental results are less than 9%. Satisfactory separation of four psoralens in temperature-programmed conditions is carried out with an analysis time of 7 min.

## Introduction

Quantitative and qualitative analysis in gas chromatography (GC) has been the subject of much debate. In isothermal GC, for example, different methods have been reported for the study of complex compound mixtures including the prediction of retention indices (1–8). Similar studies have also been conducted (9–13) in temperature-programmed GC. Generally, to study the column efficiency or the separation of a solute molecule mixture, the traditional method would be to study the initial temperature ( $T_{c,i}$ ), the final temperature ( $T_{c,f}$ ), the gradient ( $T_c$ ), and the carrier gas flow rate ( $F_c$ ) separately. If it is considered that at least 10 experiments are needed for each factor, a minimum of 40 experiments (120 if experiments are repeated three times) is necessary. The technique proposed in this paper has been employed in isothermal GC (6). This method was used with temperature-programmed GC to provide an interrelationship between the retention time of a compound, the resolution between two peaks, column plate height, initial temperature, final temperature, gradient, and carrier gas flow rate.

## Experimental

### Reagents

The chromatographed compounds were psoralen, 8-methoxy-psoralen (8-MOP) (Elder Pharmaceuticals, Paris, France),

5-methoxy-psoralen (5-MOP) (Aldrich Chemical Company, Saint Quentin Fallavier, France), and trimethoxy-psoralen (TMP) (Elder Pharmaceuticals).

### Apparatus

The analyses were performed using a Delsi DI 200 gas chromatograph equipped with a flame ionization detector (Suresne, France). A capillary DB-1 column was used (15 m × 320 μm) with a stationary phase of polymethyl siloxane and a film thickness of 1.5 μm (J&W Scientific, Folsom, CA). The carrier gas flow rate varied from 0.8 mL/min to 3.5 mL/min. In isothermal GC, the temperature varied from 154°C to 180°C. In temperature-programmed GC, the initial temperature varied from 100°C to 210°C, the final temperature varied from 230°C to 290°C, and the gradient varied from 5°C/min to 30°C/min.

### Chemometric methodology

The chemometric methodology was based on factorial designs. For two factors studied at two levels, 2<sup>2</sup> combinations

Table I. Two-Order Experimental Design for Four Factors

Experiment no.	$x_1$	$x_2$	$x_3$	$x_4$
1	1	1	1	-1
2	-1	1	1	1
3	-1	-1	1	1
4	1	-1	-1	1
5	-1	1	-1	-1
6	1	-1	1	-1
7	1	1	-1	1
8	-1	-1	-1	-1
9	0	0	0	0
10	-1	0	-1	1
11	1	-1	0	-1
12	-1	0	1	-1
13	1	0	1	1
14	0	-1	-1	0
15	0	-1	0	1
16	0	-1	1	-1
17	-1	-1	1	0
18	1	0	-1	-1

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exist. Two-level factorial designs (14) allow for the fitting of a first order (linear model) to the data (15). In general, central designs are constructed from a total of  $2^n + 2n + 1$  factor combinations, where  $n$  is the number of factors under study (16). Thus, the number of experiments required for two variables is nine. For four variables, the number is 25. This was too high, so an experimental design was developed that did not diverge from optimal properties (i.e., the independence of effect estimations and the minimization of the bias errors of the model).

The following design structure was adopted. The first design fraction was constructed with a factorial design at two levels (i.e., eight experiments). The second fraction was built up by using one experiment in the center of the experimental design. The third fraction was developed by using the Fedorov exchange method. Among experiments of a complete factorial design at three levels, those which minimize the generalized variance of the chosen model parameters were selected. The previously determined points were fixed (i.e., nine experiments).

The result of this construction is given in Table I. The total number of experiments was 18. These two-order experimental designs provided sufficient data for the fitting of a quadratic model to the data set. Such models are amenable to regression analysis.

For example, for two factors, the model takes the form:

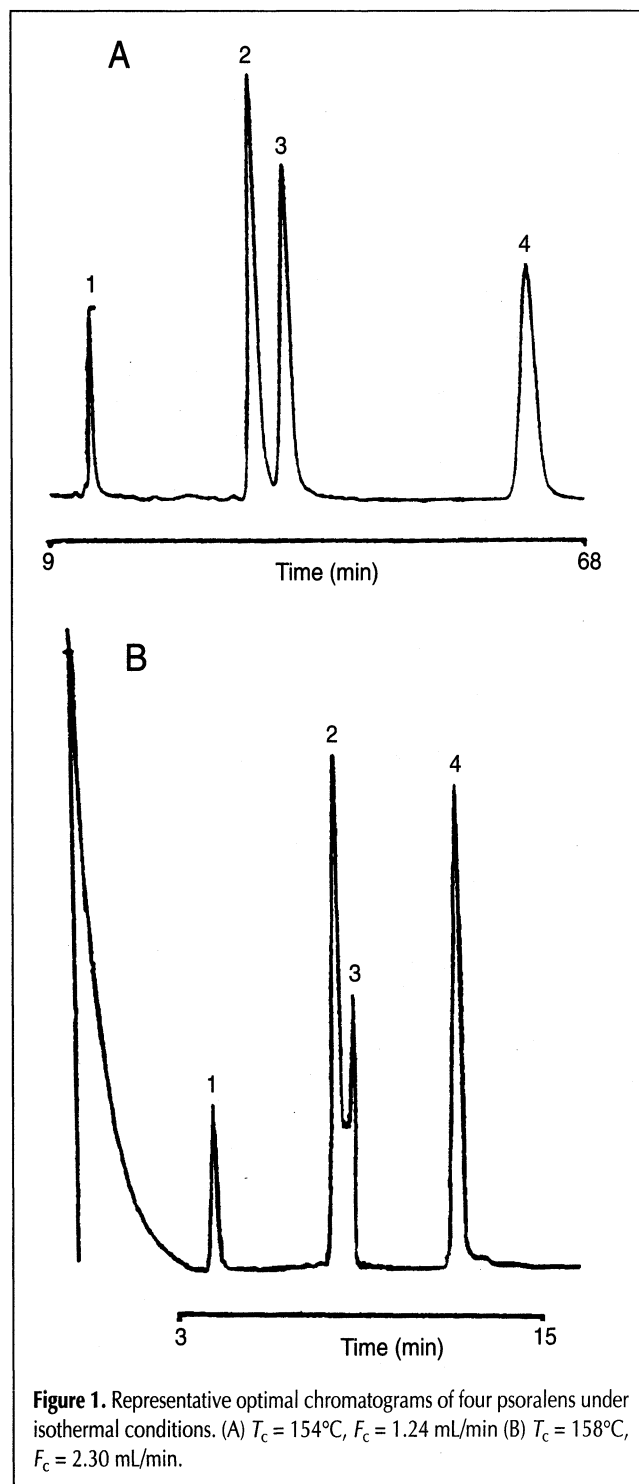
$$y = a_0 + a_1x_1 + a_2x_2 + a_{11}x_1^2 + a_{22}x_2^2 + a_{12}x_1x_2 \quad \text{Eq 1}$$

where  $y$  is the studied response, and  $x_i$  values are the logarithm of flow rate and column temperature (in isothermal condi-

tions) or the initial temperature, final temperature, and gradient (in programmed temperature conditions). The  $a_i$ ,  $a_{ij}$ , and  $a_{ij}$  terms represent the parameters of the model. All variables were coded to have a variation range from  $-\sqrt{2}$  to  $\sqrt{2}$  for two factors and from  $-1$  to  $1$  for four factors.

### Simplex optimization process

To optimize the mathematical model ( $y$ ) given in the experimental design, a simplex method was used. The  $y$  value was calculated for  $m$  sets of starting conditions where  $m$  represented the number of factors to be optimized plus one. The



**Figure 1.** Representative optimal chromatograms of four psoralens under isothermal conditions. (A)  $T_c = 154^\circ\text{C}$ ,  $F_c = 1.24$  mL/min (B)  $T_c = 158^\circ\text{C}$ ,  $F_c = 2.30$  mL/min.

**Table II. Results of the Simplex Process for  $R_s$  in Isothermal GC**

Experiment no.	$T_c$ ( $^\circ\text{C}$ )	$F_c$ (mL/min)	$R_s$
1	170	3.01	0.901
2	168	2.91	0.870
3	165	3.42	0.790
4	175	2.53	0.952
5	163	2.00	1.000
6	160	3.50	0.899
7	168	2.54	0.956
8	157	1.98	1.021
9	150	1.77	1.025
10	154	2.04	1.125
11	159	1.12	0.950
12	162	1.34	0.891
13	170	2.54	1.140
14	158	2.02	1.191
15	158	1.45	0.999
16	157	1.58	1.029
17	150	1.32	0.998
18	156	1.25	1.125
19	157	1.20	1.192
20	154	1.32	1.153
21	154	1.22	1.227
22	154	1.37	1.182
23	154	1.24	1.228
24	154	1.25	1.226
25	154	1.22	1.224

point corresponding to the lowest value of  $y$  was then reflected about the surface that was defined by the other points. A new set of starting conditions resulted. Once again, the point with the lowest value of  $y$  was reflected, and the process was repeated until the same conditions continued to be selected.

## Results and Discussion

### Isothermal GC separation

The resolution ( $R_s$ ) between two peaks is given by the equation:

$$R_s = \frac{\sqrt{N}}{4} \left( \frac{k}{1+k} \right) \left( \frac{\alpha-1}{\alpha} \right) \quad \text{Eq 2}$$

where  $N$  is the column plate number equal to  $L/H$ ,  $L$  is the column length,  $H$  is the height equivalent to a theoretical plate, and  $\alpha$  is the separation factor given by the ratio of the capacity factors  $k$  for the two compounds between which resolution is being calculated. In recent papers (5,6), it has been demonstrated in isothermal conditions that  $1/H$  (6) and  $\ln k$  (5) fit the two-order model (Equation 1) with a composite central design. Therefore, by using Equation 2,  $R_s$  can be calculated for the different values of flow rate and column temperature. This methodology was applied to the separation

of four psoralens. The column efficiency was determined with TMP solute. Nine experiments were carried out in isothermal conditions following the composite central design. The experiments were repeated three times. The variation coefficients of the  $H$  and  $k$  values were all less than 5%. Correlation coefficients of the  $\ln k$  and  $1/H$  models were all greater than or equal to 0.97. The worst separated pair of peaks was 8-MOP and 5-MOP. Using the simplex method, we found  $R_s$  to be at a maximum (1.23) when the flow rate was 1.24 mL/min and the temperature was 154°C (Table II). The analysis time was 62 min. The chromatogram is given in Figure 1A. For a routine analysis, this was too much time. To minimize the analysis time, a chromatographic response function (CRF) was used (17–21). This is defined as follows:

$$\text{CRF} = F_{\text{obj}} + n^a + b(t_A - t_L) + c(t_1 - t_2) \quad \text{Eq 3}$$

where  $F_{\text{obj}}$  is the objective function expressed in terms of the resolution factor  $R_{s_{ij}}$  between two peaks  $i$  and  $j$ . In this application,  $F_{\text{obj}}$  is given by the following:

$$F_{\text{obj}} = \sum \ln(1 + R_{s_{ij}}) \quad \text{Eq 4}$$

The sum extends to all the peak pairs on

**Table III. Results of the Simplex Process for CRF in Isothermal GC**

Experiment no.	$F_c$ (mL/min)	$T_c$ (°C)	CRF
1	1.00	161	3.61
2	1.30	157	5.91
3	1.50	171	4.33
4	1.57	152	6.55
5	1.61	140	4.05
6	1.88	159	7.27
7	2.00	180	2.39
8	2.10	174	4.75
9	2.20	164	7.09
10	2.25	161	7.48
11	2.20	156	7.48
12	1.79	152	7.05
13	1.91	155	7.33
14	2.22	167	6.64
15	2.32	157	7.51
16	2.40	165	7.05
17	2.30	158	7.52
18	2.33	162	6.86
19	2.30	155	7.43
20	2.29	157	7.50

**Table IV. Calculated ( $t_c$ ) and Measured ( $t_m$ ) Retention Time and Column Efficiency for TMP in 10 Experimental Conditions in Temperature-Programmed GC**

Experiment no.	Initial temp. $T_{c,i}$ (°C)	Final temp. $T_{c,f}$ (°C)	Gradient $T_c$ (°C/min)	Carrier gas flow rate $F_c$ (mL/min)
1	210	290	5	3.5
2	210	290	30	0.8
3	210	230	5	0.8
4	100	290	5	3.5
5	210	230	30	3.5
6	100	230	5	0.8
7	155	230	30	0.8
8	100	230	12.24	2.15
9	100	290	12.24	0.8
10	155	230	5	3.5

Experiment no.	$t_m$ (min)	$t_c$ (min)	$e$ (%)*	$H_m$ (mm)	$H_c$ (mm)	$e$ (%)*
1	5.83	5.85	0.30	0.612	0.610	0.33
2	2.03	2.05	0.98	1.000	1.040	3.85
3	10.10	10.25	1.46	0.595	0.610	2.46
4	12.13	11.90	1.89	0.119	0.127	6.30
5	5.61	5.55	1.06	0.999	1.020	2.05
6	14.10	14.25	1.06	0.255	0.269	5.20
7	7.90	7.88	0.25	0.708	0.740	4.32
8	8.15	8.20	0.61	0.215	0.221	2.71
9	9.13	9.23	1.08	0.098	0.100	2.00
10	11.12	11.24	1.07	0.302	0.287	4.96

\* Relative difference between calculated and experimental values.

the chromatogram. In Equation 3,  $n$  is the detected peak number,  $t_A$  is the maximum acceptable analysis time,  $t_L$  is the retention time of the last peak,  $t_2$  is the minimum acceptable time of the first peak, and  $t_1$  is the retention time of the first peak.

The constants  $a = 1$ ,  $b = 0.5$ , and  $c = 1$  were determined empirically to give a function that sharply discriminated unsatisfactory separations from better separations. To prevent the solvent peak from interfering with the psoralen peak,  $t_2$  was chosen as 2 min;  $t_A$  was set at 20 min. The optimum separation conditions were obtained when CRF reached its maximum in a short analysis time. The coefficient of multiple determination that corresponded to the CRF model was 0.97. The simplex optimization process resulted in a maximum CRF (7.52) for a flow rate of 2.30 mL/min and a temperature of 158°C (Table III). The corresponding analysis time was equal to 13 min, and the separation factor between 8-MOP and 5-MOP was 0.60. The chromatogram is given in Figure 1B. If this separation had been proposed only for the qualitative determination of the four compounds in the mixture, it would not have been necessary to obtain an optimal resolution. For a quantitative analysis, this value of the resolution had to be increased. The separation was then carried out in temperature-programmed GC.

### Temperature-programmed GC: Application for the separation optimization of four psoralens

The effects of flow rate and temperature parameters on solute retention time and column efficiency were studied. The experimental  $H$  and  $k$  values for each compound were calculated from the 18 chromatograms given by our constructed experimental design. All the experiments were repeated three times, making a total of 54 experiments. The variation coefficients of  $H$  and  $k$  values were all less than 5%, which indicated high reproducibility and good stability for the chromatographic system. The results were processed by computer, and the parameters of the  $\ln k$  and  $1/H$  models were obtained. The models fit the results well. All the correlation coefficients were greater than or equal to 0.97. The student T test confirmed the well-known result that the  $k$  value is not dependent on the gas flow rate. The retention time for each compound is given by the equation:

$$t = t_0(1 + k) \quad \text{Eq 5}$$

where  $t_0$  is the column dead time determined with methane or air;  $t_0$  is the time needed for the carrier gas to go through the column. For a given column,  $t_0$  is the same in isothermal and programmed-temperature GC for identical experimental conditions of flow rate. The variable  $t_0$  is given by the equation (22,23):

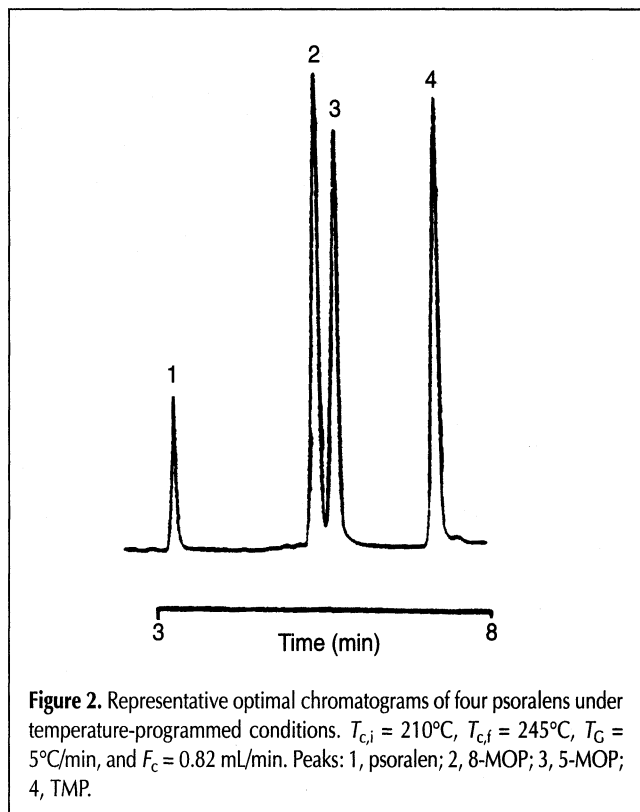
$$t_0(\text{min}) = \frac{\phi}{F_c(\text{mL/min})} \quad \text{Eq 6}$$

The method used to determine the constant  $\phi = 1.81$  was detailed in a previous paper (6). Knowing the  $\phi$  value and the variation of  $\ln k$  with the four factors, the retention time  $t$  for the TMP solute was calculated for 10 experimental conditions (Table IV). The observed agreement between predicted and experimental values showed the suitability of the models.

For  $1/H$  models, the student T test showed that all the factors were significant. The predicted and measured  $H$  values for 10 experiments are given in Table IV. Using Equation 2, we calculated the resolution between two peaks for the different values of the four factors (flow rate, initial temperature, final temperature, and gradient). The maximum  $R_s$  (1.03) was determined with the simplex method and exhibited the highest resolution for the worst separated pair of peaks, which was the same as in isothermal conditions (i.e., 8-MOP and 5-MOP). The optimum separation was obtained in 7 min with a flow rate of 0.82 mL/min and initial, final, and gradient temperatures equal to 210°C, 245°C, and 5°C/min, respectively (Table V). The analysis time was thus reduced by 90% (7 min

Table V. Results of the Simplex Process for  $R_s$  in Temperature-Programmed GC

Experiment no.	$F_c$ (mL/min)	$T_{c,i}$ (°C)	$T_{c,f}$ (°C)	$T_G$ (°C/min)	$R_s$
1	0.82	100	230	25.00	0.250
2	0.84	125	240	15.00	0.321
3	3.50	210	225	30.00	0.112
4	2.82	150	220	12.22	0.212
5	1.44	172	260	20.45	0.099
6	1.69	184	270	25.58	0.088
7	1.23	175	275	27.89	0.110
8	2.45	190	285	15.12	0.432
9	2.25	182	270	10.75	0.655
10	3.40	185	235	12.45	0.555
11	2.50	125	240	18.76	0.741
12	1.56	160	235	16.45	0.811
13	1.42	165	243	14.28	0.611
14	2.02	189	261	18.41	0.588
15	1.45	185	259	15.21	0.788
16	1.25	181	261	11.30	0.981
17	1.12	200	255	15.23	0.881
18	1.25	205	264	14.42	0.774
19	0.92	216	257	11.39	0.992
20	0.81	205	255	9.41	0.997
21	0.75	211	258	7.25	1.001
22	0.82	195	248	10.31	0.842
23	0.82	210	245	5.00	1.031
24	0.82	196	246	5.23	0.999
25	0.82	207	254	5.00	1.012
26	0.92	205	255	7.10	1.002
27	0.90	211	246	8.04	0.888
28	0.86	195	247	6.52	1.002
29	0.82	210	245	5.00	1.015
30	0.87	210	245	5.00	1.011



instead of 62 min in isothermal conditions). The chromatogram is given in Figure 2. The results confirmed the method used by Yan (24) for the quantitative determination of psoralens in pharmaceuticals with an analysis time of 12 min. These new separation conditions decreased analysis time from 12 min to 7 min.

## Conclusion

The results of this study demonstrate that this method can be used in GC for modeling solute retention time and a compound mixture separation both in isothermal and temperature-programmed conditions. This procedure reduces the number of experiments to be carried out and shows the utility of using programmed temperatures instead of isothermal GC for rapid separation.

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