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Prediction of gas chromatographic retention times, column efficiency and resolution as a function of temperature and flow-rate

Application for gas chromatographic separation of eight *p*-hydroxybenzoic esters

Y. Guillaume, M. Thomassin, C. Guinchard*

Laboratoire de Chimie Analytique, UFR des Sciences Médicales et Pharmaceutiques, 25030 Besançon cedex, France

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Abstract

Using eight *p*-hydroxybenzoic esters, simple equations were developed to provide accurate gas chromatographic retention data and column efficiency represented by the height equivalent to a theoretical plate. With an experimental design, these equations have practical applications in the calculation of the resolution for a given pair of these compounds and for the determination of the isothermal conditions for their separation in minimum time. The method reduced the number of experiments to be carried out and provided good agreement between experimental results and predicted data.

1. Introduction

Qualitative and quantitative analysis in GC has been the subject of much debate. A paper listing some 900 Kovats indices for 400 monoterpenes on methylsilicone and Carbowax 20 M phases has been published [1].

Different methods have been reported for the determination of the universal retention index [2]. A procedure has been described that allowed the calculation of linear temperature-programmed retention indices from Kovats retention indices on a given stationary phase [3]. The use of the UNIFAC group contribution method to predict retention on low-molecular-mass

stationary phases [4] and on polymer phases [5] was studied. Peng et al. discussed the prediction of retention indices for silylated derivatives of polar compounds [6]. Several studies examined the use of cubic splines to calculate temperature-programmed retention indices [7–9].

Fernandez-Sanchez et al. tested several methods to calculate programmed-temperature retention indices and preferred the cubic spline approach [10]. Prediction of retention data and/or structure–retention correlations were presented for the C₆–C₁₂ alkylbenzenes [11], polychlorobenzenes [12], chlorinated dibenzofuranes [13], bromo and bromo-chloro dioxins and furans [14], anabolic steroids [15], and for stimulants and narcotics [16].

Germino and Castello reported the GC identi-

* Corresponding author.

fication of complex mixtures of halomethanes and haloethanes by correlating retention with vapor pressure [17]. Practical guidelines for generating a simulated gas chromatogram and a new approach for the chromatographic indexing of organic compounds were presented by Mowery [18]. Jorgensen et al. described a model that could be used to accurately predict GC–flame ionization detector response factors from molecular structure [19].

Guillaume and Guinchard, using a molecular connectivity index, introduced a method for predicting retention indices for a series of *p*-hydroxybenzoic esters [20]. The use of chemometric methodology for the optimization of GC performance has been reported in several studies. Bautz et al. described the performance of a computer method to predict retention bandwidth, and resolution for programmed-temperature GC separations with an average error of better than 10% [21].

Dolan et al. investigated the changes on band spacing as a function of temperature through computer simulation [22]. A simplex method was used to optimize selectivity for the separation of 10 compounds by GC [23] and for the deconvolution of complex GC profiles [24].

This paper shows the interrelationships between the retention time of a *p*-hydroxybenzoic ester, its molecular connectivity index, the resolution between two solutes, column plate-height, carrier gas flow-rate and column temperature. To show this relationship, an experimental design has given suitable retention models. Thus the number of experiments used for the optimization of the separation of eight esters could be reduced with this chemometric methodology developed in our laboratory.

2. Experimental

2.1. Reagents

The chromatographed compounds were *p*-hydroxybenzoic esters. The straight chain esters, methyl to butyl (MetR, EtR, PrR, BuR) were purchased from Interchim (Montluçon, France).

The branch chain esters isopropyl, isobutyl, *sec*-butyl and *tert*-butyl (IprR, IbuR, SbuR, TerR) were synthesized in our laboratory [20].

2.2. Apparatus

The analyses were performed using a DELSI DI200 gas chromatograph with a flame-ionization detector (Suresnes, France) and a Merck D2500 chromato-integrator (Nogent-sur-Marne, France).

A capillary column OV17 was used with a stationary phase of polymethylsiloxane (25 m × 300 μm I.D.) with a film thickness of 0.2 μm (Spiral, Dijon, France). The carrier gas was nitrogen with a flow-rate varying from 1.50 to 3 ml/min and the column temperature was varied from 145°C to 170°C with isothermal conditions.

2.3. Chemometric methodology

The chemometric approach is based on factorial designs. These are conveniently defined as a base, representing the number of levels of the factors, raised to an exponent, representing the number of factors under study. Thus in the general case of k factors at two levels, 2^k factor combinations exist [25]. Two-level factorial designs allow for the fitting of a first order (linear) model to the data. If the effects of each factor do not vary linearly, a design which requires fewer experiments to detect curvature in the response is the composite central design developed by Box and Wilson [26]. The composite central design was developed specifically to enable a second-order response surface to be fitted to the data. An example of a two-factor factorial design is shown in Table 1, which illustrates the addition of levels to a 2^k factorial design. In general central composite designs are constructed of a total of $2^k + 2k + 1$ factor combinations [27] where k is the number of factors under study. Thus the number of experiments required for two variables is 9.

Composite central designs provide sufficient data for the fitting of a quadratic model to a data set. Such models are amenable to regression analysis. For two factors this takes the form:

Table 1
Experiments required for a two variable experimental design

Experiment N ^o	x_1	x_2
1	-1	-1
2	+1	-1
3	-1	+1
4	+1	+1
5	$-\sqrt{2}$	0
6	$+\sqrt{2}$	0
7	0	$-\sqrt{2}$
8	0	$+\sqrt{2}$
9	0	0

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

where y is the response or dependent variable and x_1 , x_2 are the logarithm respectively of column temperature T (°C) and carrier gas flow-rate F (ml/min). The a terms represent the parameters of the model.

3. Results and discussion

3.1. Height equivalent to a theoretical plate number study

A parameter that affects column efficiency is the average linear gas velocity (u):

$$u = \frac{L}{t_0} \quad (2)$$

where L is the column length and t_0 the retention time of an unretained peak such as air or methane. The linear velocities encountered in capillary GC normally range from ≈ 10 to >100 cm/s.

The flow-rate at the column exit, F_C^o relates to the average linear velocity by

$$F_C^o = [(u\pi r_C^2)/j] (T_0/T) \quad (3)$$

where r_C (column radius), T_0 (room temperature) and T (column temperature) are included to adjust the column outlet flow for measurement at room temperature. The constant j is a

gas compressibility factor that compensates for the change in pressure across the column length.

Although often neglected, j has a significant effect at column head pressures greater than ≈ 5 psi. Using the experimental design, t_0 was modelled by the two-order polynomial (Eq. 1) where y is equal to t_0 . From the full regression model (Table 2) a student T-test was used to provide the basis for a decision as to whether or not the model coefficients were significant. Results of the student t -test show that all the variables except x_2 and x_2^2 can be eliminated from the model; t_0 is then given by the equation:

$$t_0(\text{min}) = 0.890 - 0.223x_2 + 0.051x_2^2 \quad (4)$$

This generated model was assessed statistically using a Fischer Snedecor test and a coefficient of multiple determination R^2 . The validity of the model was tested by a Fischer ratio and the value of R^2 was used as an indicator of the explanatory power and assumed a value from 0 to +1 indicating that these variables in the model perfectly explained the variation in the independent variable t_0 .

The Fischer ratio was 6230 and 0.999 was R^2 . These values show the excellent validity of the model. The predicted and measured t_0 values for seven experiments are given in Table 3. The retention of the second order term x_2^2 of the model showed that the flow-rate (F) influenced the degree of curvature of the curve. For a

Table 2
Estimates of regression parameters for the two regression models

Independent variables	Parameter terms	N/L^a	t_0^b
Intercept	a_0	+0.726	+0.890
x_1	a_1	-0.066	-0.006
x_2	a_2	-0.046	-0.223
x_1^2	a_{11}	-0.116	+0.010
x_2^2	a_{22}	-0.047	+0.051
x_1x_2	a_{12}	-0.051	+0.002

^a Reverse of the height to a theoretical plate (mm^{-1}).

^b Column dead time (min).

Table 3

Predicted (p) and measured (m) values of the column dead time t_0 (min) and the reverse of the plate height N/L (mm^{-1}) for different experimental conditions

Experiment No.	Linear velocity (cm/s)	Temperature (°C)	t_0^p	t_0^m	Δt_0 (%)	N^p/L	N^m/L	$\Delta(N/L)$ (%)
1	62.00	170.0	0.67	0.68	1.47	0.14	0.13	7.14
2	62.00	160.0	0.67	0.67	0.00	0.51	0.48	5.88
3	62.00	145.0	0.67	0.68	1.47	0.54	0.51	5.88
4	32.00	170.0	1.30	1.31	0.76	0.47	0.45	4.44
6	32.00	160.0	1.30	1.30	0.00	0.69	0.67	2.89
6	32.00	145.0	1.30	1.30	0.00	0.48	0.45	6.20
7	46.00	145.0	0.91	0.91	0.00	0.59	0.54	5.08

flow-rate ranging from 1.5 to 3 ml/min, the corresponding dead time t_0 was plotted on the y -axis (Fig. 1). The slight curvature in the plots is caused by the compressibility factor j . Eq. (4) can be written as the well known equation [28,29]

$$t_0(\text{min}) = \frac{\Phi_1}{F(\text{ml/min})} \quad (5)$$

where Φ_1 is a constant calculated to be 2.02. Combining Eqs. (2) and (5) the following is obtained

$$u(\text{cm/s}) = \Phi_2 F(\text{ml/min}) \quad (6)$$

where Φ_2 is equal to 20.70.

A similar method was used to study column dead time in reversed-phase chromatography [30].

In gas chromatography, the retention time of a solute molecule is given by the equation:

$$t = t_0(1 + k') \quad (7)$$

For this series of eight p -hydroxybenzoic esters, k' was described by the following equation [20]:

$$\ln k' = {}^1\chi \left(\frac{\epsilon}{T} + \Phi \right) + \frac{\gamma}{T} + \delta \quad (8)$$

where ϵ , Φ , γ and δ are constants, T (K) is the column temperature and ${}^1\chi$ the ester molecular connectivity index [20].

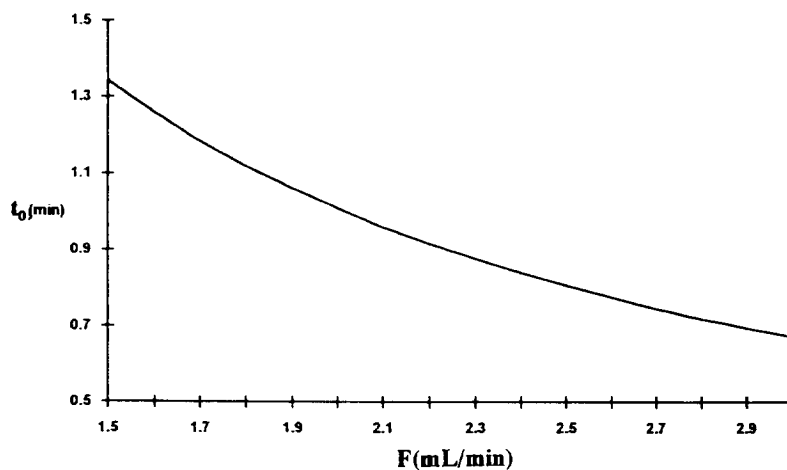


Fig. 1. Plot of column dead time vs. flow-rate.

Table 4
Predicted (t^p) and measured (t^m) retention time for the eight esters

Compound ^a No.	[I]				[II]			
	t_1^p (min)	t_1^m (min)	Δt_1 (min)	Δt_1 (%)	t_{II}^p (min)	t_{II}^m (min)	Δt_{II} (min)	Δt_{II} (%)
MeR = 1	10.11	9.74	0.37	3.70	2.22	2.21	0.01	0.45
EtR = 2	14.07	13.54	0.53	3.74	2.81	2.79	0.02	0.71
IprR = 3	17.65	16.94	0.71	4.00	3.33	3.33	0.00	0.00
PrR = 4	18.77	18.00	0.77	4.10	3.49	3.48	0.01	0.29
TerR = 5	21.23	20.19	1.04	4.89	3.84	3.82	0.02	0.52
IbuR = 6	23.17	21.99	1.18	5.10	4.10	3.99	0.02	0.27
SbuR = 7	24.25	23.06	1.19	4.90	4.25	4.22	0.03	0.71
BuR = 8	25.00	23.72	1.28	5.12	4.39	4.32	0.07	0.16

^a 1 = Methyl parahydroxybenzoic ester, 2 = ethyl parahydroxybenzoic ester, 3 = isopropyl parahydroxybenzoic ester, 4 = propyl parahydroxybenzoic ester, 5 = tertbutyl parahydroxybenzoic ester, 6 = isobutyl parahydroxybenzoic ester, 7 = secbutyl parahydroxybenzoic ester, 8 = butyl parahydroxybenzoic ester.

Conditions: [I] Temperature = 145°C, flow-rate = 1.55 ml/min; [II] Temperature = 170°C, flow-rate = 3.00 ml/min.

Combining Eqs. (5), (7) and (8), a simple empirical equation was found relating the retention time t to the column temperature T (K) and gas flow-rate F (ml/min).

$$t = \frac{\Phi_1}{F} \left(1 + \exp \left(\chi \left(\frac{\epsilon}{T} + \Phi \right) + \frac{\gamma}{T} + \delta \right) \right) \quad (9)$$

Since the constant Φ_1 and the connectivity index

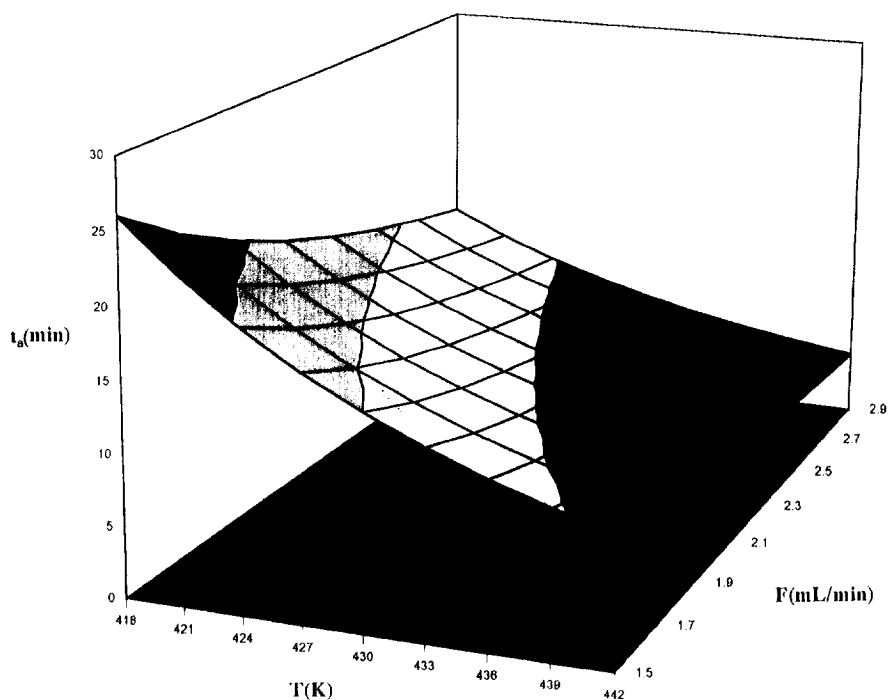
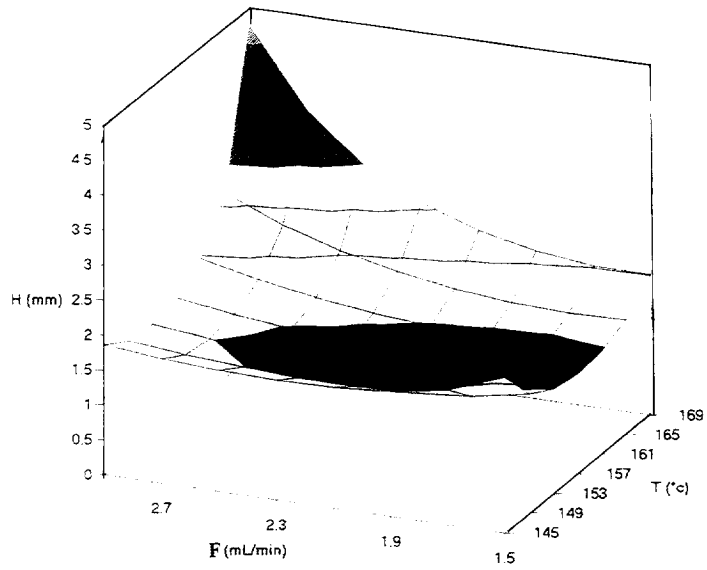


Fig. 2. Response surface for the analysis time t_a vs. F and T .

A



B

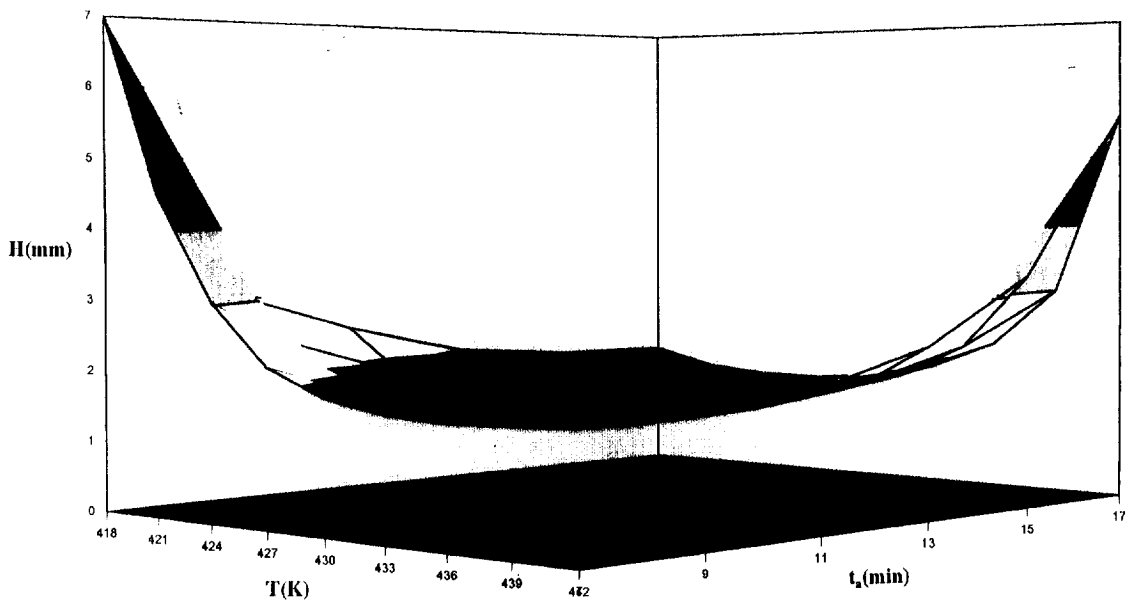


Fig. 3. Response surface of the height to a theoretical plate: (A) vs. column temperature/carrier gas flow-rate; (B) vs. analysis time/column temperature; (C) vs. analysis time/carrier gas flow-rate.

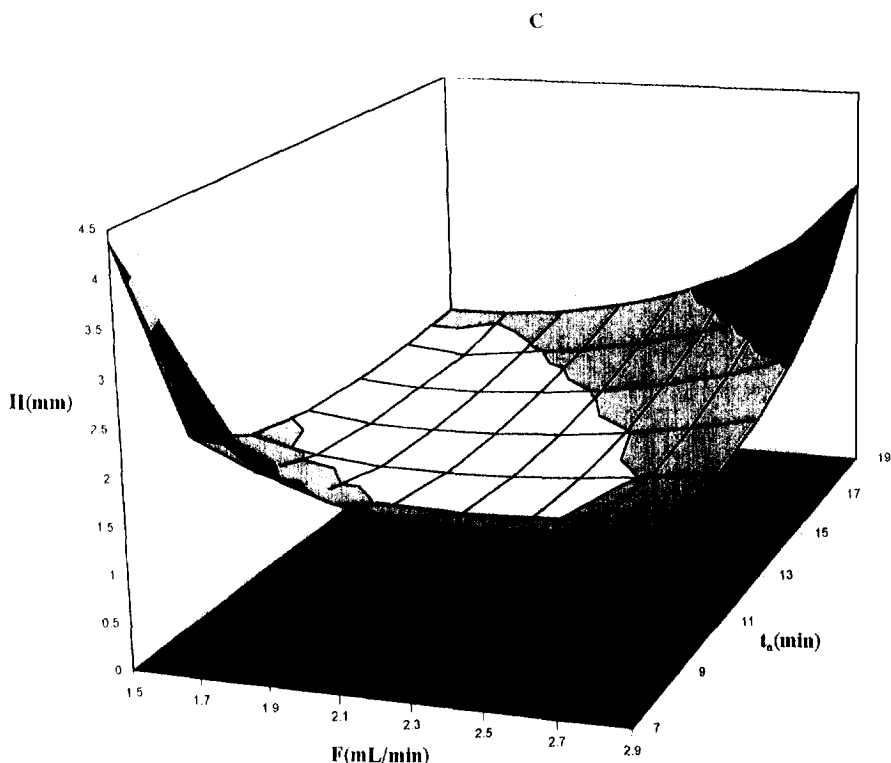


Fig. 3 (Continued).

χ^2 of the eight compounds [20] were known, their retention times t were calculated in accordance with the following experimental conditions: [I] $F = 1.55$ ml/min, $T = 145^\circ\text{C}$; [II] $F = 3.00$ ml/min, $T = 170^\circ\text{C}$.

The data for [I] varied as expected, i.e., a larger error at longer retention times. The good agreement observed between the predicted and experimental values shows the suitability of the model (Table 4). The response surface generated for the analysis time (t_a) model (Eq. 9) is given in Fig. 2 for the last compound on the chromatogram (butyl-*p*-hydroxy-benzoic ester).

In gas chromatography, the height equivalent to a theoretical plate (H) is used to study the sharpness of a peak. H depends on the carrier gas flow-rate and column temperature. H is given by the following equation:

$$H = \frac{L}{5.54} \left(\frac{W_{0.5}}{t} \right)^2 \quad (10)$$

where t is the retention time and $W_{0.5}$ the peak-width at half height. The corresponding column plate number (N) is:

$$N = \frac{L}{H} = 5.54 \left(\frac{t}{W_{0.5}} \right)^2 \quad (11)$$

The parameters estimates generated for the regression N/L model are given in Table 2.

The values of the Fischer ratio and R^2 were 80.40 and 0.974, respectively. The results of the student t -test show that all the factors are significant. The predicted and measured N/L values for seven experiments are given in Table 3. The response surface generated for the H -model is given in Fig. 3A. Using Eq. (9) relating separation analysis time t_a to carrier gas flow-rate F and column temperature T , two other response surfaces for the H -model are obtained (Figs. 3B,C). A similar chemometric methodology was used to study column efficiency in reversed-phase chromatography [30].

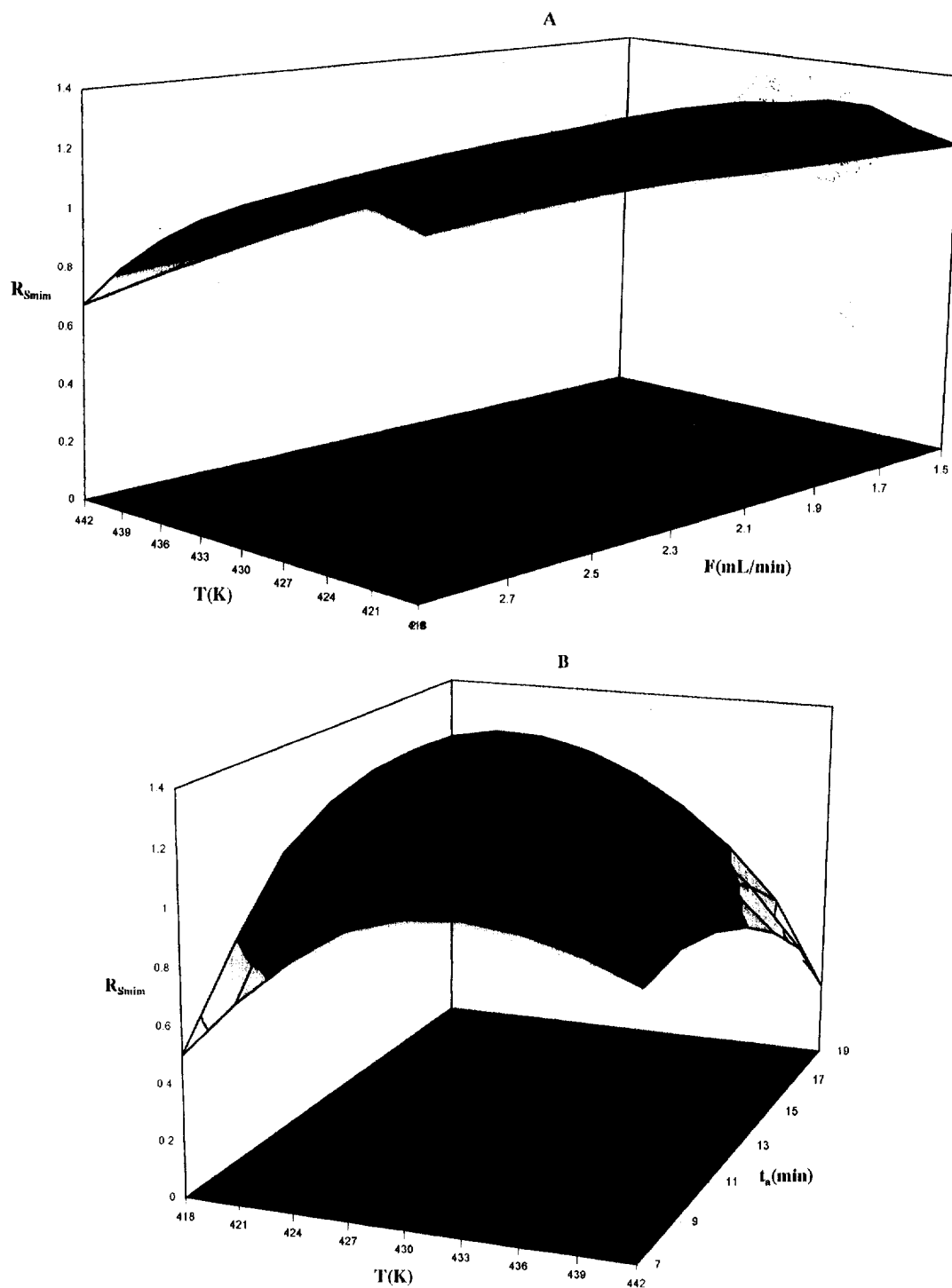


Fig. 4. Response surface of the minimal resolution R_s min: (A) vs. column temperature/carrier gas flow-rate; (B) vs. analysis time/column temperature (C) vs. analysis time/carrier gas flow-rate.

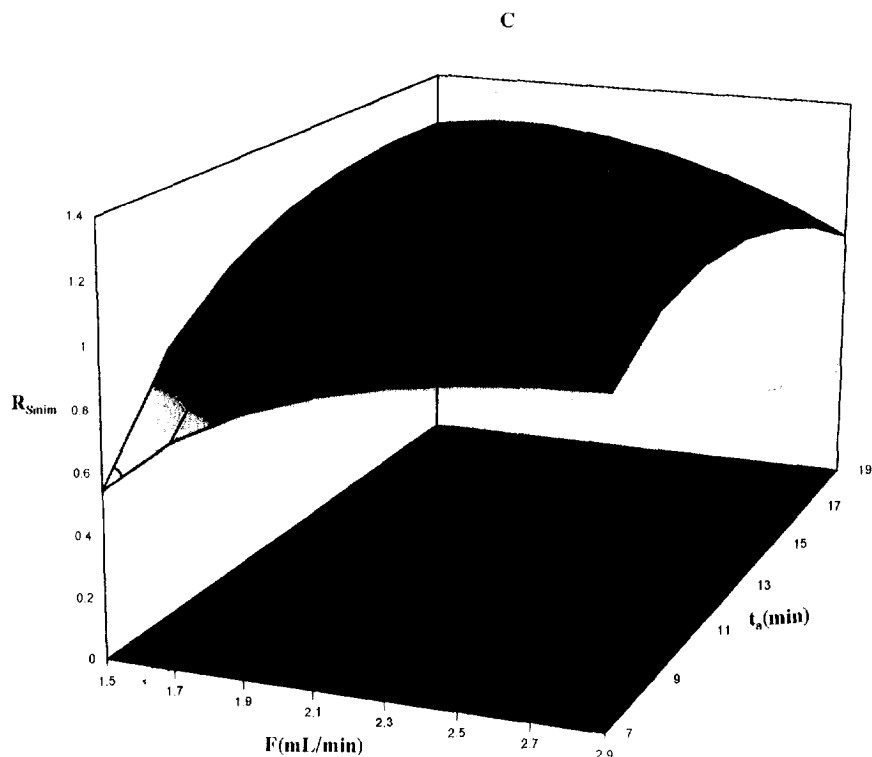


Fig. 4 (Continued).

Obviously, optimum column temperature and carrier gas flow-rate are minimum according respectively to the equation of De Wett and Pretorius [31] and Van Deemter et al. [32] which relate respectively H to the column temperature and gas flow-rate. Further improvements and investigations made by others [33–41] do not take into account the simultaneous variation of F and T . This chemometric methodology provided an equation relating H to both gas velocity and temperature. The partial derivatives of the H -model against x_1 and x_2 were equal to zero and the optimal temperature and gas flow-rate were determined and found to be equal to 156°C and 1.90 ml/min. Figs. 3B and 3C present a global optimum, i.e., the maximal efficiency was obtained for a single combination of analysis time/gas flow-rate or analysis time/column temperature. In our case for optimal temperature and gas flow-rate, the optimum analysis time was calculated to be 12.00 min. The experimental

optimum value of t_a was 12.60 min. The theoretical value was good.

3.2. Eight compound separation study

Whatever the factor variation, all compounds were arranged on a chromatogram in the same order. The resolution (R_s) between two adjacent peaks can be affected by the three variables:

$$R_s = \frac{\sqrt{N}}{4} \left(\frac{k'}{1+k'} \right) \left(\frac{\alpha-1}{\alpha} \right) \quad (12)$$

where α is the separation factor given by the ratio of the capacity factor for the two solutes between which resolution is being calculated. Computer simulations have begun to play an increasing role in the optimization of separations and in the understanding of retention data [21–24].

The interest of this method used here is that it takes into account the analyses time t_a and the

simultaneous variation of column efficiency with the two factors x_1 and x_2 . The experimental design reduced the number of experiments to be carried out. Therefore, knowing the variation of N , k' and α with x_1 and x_2 , R_s can be calculated using Eq. (12) for the different values of the two factors. The highest resolution for the worst separated pair of peaks (R_s min) (*sec*-butyl, butyl-*p*-hydroxy-benzoic esters) was used as criterion of separation.

Using Eqs. (9) and (12) there are three response surfaces generated for the R_s min model (Fig. 4A,B,C). The maximum R_s (1.260) was the highest for the worst separated pair of peaks. The optimum conditions were a gas flow-rate of 2.1 ml/min with a column temperature equal to 424 K with an analysis time of 13.20

min. The corresponding chromatogram is given in Fig. 5A.

Obviously, the objective must be to minimize the analysis time and not be too demanding with respect to resolution. If R_s min was chosen to be equal to 1.10 for an analysis time of 8.00 min, Figs. 4B and 4C show that gas flow-rate and column temperature were respectively equal to 2.90 ml/min and 430 K. The corresponding chromatogram is given in Fig. 5B. It can be noted that column efficiency and optimum separation conditions were similar only in relation to the column temperature.

4. Conclusion

For a convenient way of ascertaining column temperature and gas flow-rate, this method which both reduces the number of experiments to be carried out and provides suitable retention models, is an attractive approach for optimization in GC.

This optimization procedure could be of interest to industrial process and control engineers as it both reduces analysis time and improves separation.

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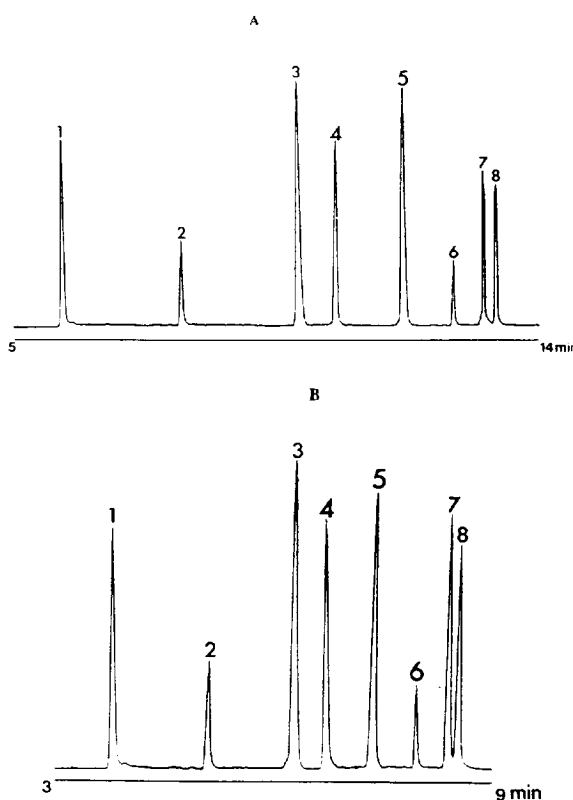


Fig. 5. *p*-Hydroxybenzoic ester chromatogram in the conditions: (A) $F = 2.1$ ml/min, $T = 424$ K; (B) $F = 2.9$ ml/min, $T = 430$ K. Number above peaks refers to the eight compounds: see Table 4.

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