

Optimization of an ion-exchange high-performance liquid chromatographic method for the determination of carboxylic acids, sugars, glycerol and ethanol in wines

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ABSTRACT

Optimization of ion-exchange high-performance liquid chromatography with refractive index detection of major carboxylic acids, sugars, glycerol and ethanol in wines was carried out by using a super-modified simplex method and a modelling response surface method. A modification of the CRF criterion (chromatographic response function) for selectivity optimization was used, including different weighting factors for each pair of peaks (VCP criterion; variable coefficient parameters).

INTRODUCTION

The traditional method for the determination of carboxylic acids involves individual enzymatic analysis, but is time consuming and tedious. To overcome these drawbacks, high-performance liquid chromatography (HPLC) has recently been applied to the determination of organic acids in wines.

Several HPLC methods are in current use including reversed-phase chromatography [1–12] and ion-exchange chromatography with either strong cation- or weak anion-exchange resins [13–18]. Two principal methods are based on the first technique, the direct method, with detection at 210 nm [2,3,9, 11] or refractive index detection [1,10], and the derivatization method, using different reagents such as phenacyl [5,6,12], *p*-nitrophenyl [4] or *p*-nitrobenzyl [7] bromide with detection at 254 nm.

When ion chromatography is used, separation is usually carried out with a polymeric cation exchanger in the hydrogen form, where the primary mechanism for the separation of acids is ion exclusion at the pH used. Detection is carried out with a UV detector [13,14,17] in order to eliminate the

interference from sugar, or with an IR detector [13,17,18], which can only be applied to dry wines.

When ion-exclusion HPLC with IR detection is applied to a dry wine, the method determines major carboxylic acids, most sugars (glucose and fructose), glycerol and ethanol, all of them important enological parameters, very rapidly. For the simultaneous determination of major carboxylic acids, sugars, glycerol and ethanol in wines, different mobile phases (dilute acids), column temperatures and flow-rates have been used by several workers [13,14, 16–18]. The aim of this study was to optimize the chromatographic conditions for the determination of these compounds.

Two main approaches have been described for the optimization process, sequential and simultaneous techniques [19]. Both have been used in HPLC optimization and their advantages and drawbacks have been described [20–21]. The super-modified sequential simplex method [23,24] and the simultaneous modelling of the response surface method [20,21] were used to optimize the chromatographic parameters, pH and flow-rate of the mobile phase and column temperature.

A computer program allows us to select the

model, compute the coefficients from the experimental data and, after validation of the selected equation, indicate the experimental conditions corresponding to the best response, and to display the response surface calculated. The parameter space chosen to model the response surface was the same as that used for the simplex method.

To carry out the optimization method, it is necessary to define the criterion to be maximized [21,22]. First, the *CRF* criterion (chromatographic response function) was used, but the characteristics of this analysis forced us to modify this criterion and introduce a new criterion, *VCP* (variable coefficient parameters), with weighting factors for each pair of peaks in the resolution term and the number of peaks appearing in the chromatogram.

EXPERIMENTAL

Equipment

A Shimadzu Model LC-9A pump and a Shimadzu RID-6A detector were used. The chromatographic separation was carried out with an ION-300 (300 × 7.8 mm I.D.) column containing a polymeric cation exchanger in the hydrogen form, with an Ion Guard GC-801 column (Interaction).

Reagents and standards

Standard solutions of the major carboxylic acids found in wine (tartaric, lactic, malic, acetic, citric and succinic acids) (Aldrich), glucose, fructose, glycerol and ethanol (all of analytical-reagent grade; Merck) were prepared in water to provide a concentration of 250 ppm, except for the ethanol, which was prepared at 500 ppm.

The mobile phase was prepared with sulphuric acid (Merck) at different concentrations. All solutions were prepared with ultra-pure water obtained with a Milli-Q purification system (Millipore). All standards and solvents were filtered with 0.45- μ m membrane.

RESULTS AND DISCUSSION

To carry out the optimization process it was necessary to define the variables to be optimized and their parameter spaces. The temperature of the column, the concentration of the sulphuric acid in the mobile phase and the flow-rate were chosen. The

parameter spaces were determined from bibliographic data and the instrumental limitations.

The range of temperatures chosen was between 30°C (10°C higher than room temperature) and 80°C (maximum temperature recommended for the column used). The recommended eluent for the column was sulphuric acid diluted to between 0.01 and 0.05 *N*. Other strong acids, such as perchloric and nitric acid, can be used, but halide-containing acids, such as hydrochloric acid, are not recommended owing to their corrosive effect on stainless steel. The recommended eluent flow-rate for the ION-300 column is 0.1–0.6 ml/min. Higher flow-rates are not possible because of the increase in the pressure of the column, and for the ION-300 150 atm must not be exceeded.

When the parameter space had been defined, the super-modified simplex method and the modelling surface response method were used to determine the optimum conditions.

Simplex method

The sequential super-modified simplex method [23,24] was used. This version offers some advantages over the modified simplex method [25]. It has the ability to change its size and orient itself to fit a response surface by second-order and Gaussian estimation of the position of an optimum vertex from the previously obtained response. The criterion used to stop the simplex optimization was the coefficient of variation (COV), the relative standard deviation of the response, in the simplex expressed as a percentage of the mean response.

Before applying the method, it was necessary to determine the criterion to be maximized. In the present work the *CRF* criterion was selected [20], and the expression used was:

$$CRF = a \sum_{i=1}^n (R_{i+1,i}) + b(t_m - t_1)$$

where *a* and *b* are the weighting factors, *R_i* the resolution value between two adjacent peaks, *t_m* is the maximum desired analysis time and *t₁* the retention time of the last peak.

If the same importance is given to two terms, the weighting factors have to be *a* = *b* = 1. In this instance the simplex method goes to conditions with a low COV value (0.58%) after 22 experiments, but under these conditions a poor resolution was obtain-

ed for a pair of peaks. Under these conditions, only one peak appears for malic acid and fructose whereas good resolution between the remainder of the peaks can be observed. In this criterion the resolution values between peaks 5–6, 8–9 and 9–10 were not taken into account in the optimization process because peak pairs with a resolution higher than 3 for all the experimental conditions have not been considered. When very different values of the resolution are obtained in the chromatogram, it is necessary not to consider the best resolved peaks in order to increase the importance of the resolution values corresponding to the less resolved peaks in the global criterion.

In order to increase the importance of the resolution term compared with the analysis time term, some different weighting factor values were studied and finally of $a = 1$ and $b = 0.3$ were considered in the CRF criterion. Under the optimum conditions, the resolution between fructose and malic acid improves at the expense of analysis time, but good resolution is not yet obtained.

Ten peaks appear in the optimization analysis, three of which have poor resolution (glucose, malic acid and fructose), depending on the experimental conditions; sometimes the worst resolution was between glucose and malic acid and sometimes it was between malic acid and fructose and the ethanol peak with a high retention time and very separated from the remainder of the peaks. In this instance, greater importance was given to increasing the resolution between these two badly resolved peaks than was given to other peaks.

To increase the importance of the resolution term between the different peaks, a new coefficient was introduced in the CRF expression, similar to that used by Glajch *et al.* [26] in the COF criterion.

$$\text{COF} = \sum_{i=1}^n a_i \ln(R_i/R_{i,d}) + b(t_m - t_1)$$

where R_i is the resolution for the i th pair of peaks, $R_{i,d}$ is the desired resolution for the i th pair and a_i is a weighting parameter for each pair of peaks.

The weighting factor c_i was included such that the separation between each pair of peaks could be made larger or smaller. On the other hand, another term for the number of peaks (n) is introduced to force the appearance of the maximum number of peaks in the chromatogram.

The global criterion VCP including the previous mentioned modification is expressed as:

$$VCP = a \sum_{i=1}^n c_i R_{i+1,i} + b(t_m - t_1) + n$$

This criterion implies the identification of each peak in the chromatogram and the absence of crossovers.

This criterion was used to develop the simplex method with values of the weighting factors of $a = 1$ and $b = 0.6$ (to increase the importance of the resolution term compared with the time term); coefficient values of $c_i = 3$ for the pairs of peaks 2–3, 3–4 and 4–5, $c_i = 1$ for the pairs of peaks 1–2, 6–7 and 7–8 and $c_i = 0$ for the pairs of peaks 5–6, 8–9 and 9–10 were chosen for this experiment. The maximum analysis time was established as 25 min.

Under these conditions the simplex method was developed and after eighteen experiments (Table I) (COV 1.2%) the optimum conditions were obtained in experiment number 15 as follows: concentration of the sulphuric acid in the mobile phase 0.01 N , temperature of the column 71°C and flow-rate 0.572 ml/min.

In Fig. 1 the simplex movements can be observed. The initial simplex is shown as a dotted line, and the lines describe the different movements (the contractions are not shown). The different experimental points quickly lead to a low value of the concentration of the sulphuric acid in the mobile phase, and then the different movements lead to a high value of the variables y and z , temperature and flow-rate, respectively.

The chromatogram obtained under these conditions is shown in Fig. 2, where the best resolution between the peaks of the glucose, malic acid and fructose can be observed with an analysis time of less than 25 min.

Modelling response surface method

This simultaneous method allows the optimization of different experimental parameters knowing the influence of each parameter on the response. The parameter space chosen to model the response surface was the same as that used for the simplex method. The experimental design shown in Fig. 3 was selected in this study in order to obtain a correct modelling of the response surface.

A third-order linear model with interaction was

TABLE I

EXPERIMENTAL POINTS WHEN THE SIMPLEX METHOD IS DEVELOPED USING THE *VCP* CRITERION

Vertex No.	Variable			Response (<i>VCP</i>)	COV (%)
	H ₂ SO ₄ concentration (<i>N</i>)	Temperature (°C)	Flow-rate (ml/min)		
1	0.010	30	0.300	1.93	
2	0.048	42	0.371	9.97	
3	0.019	77	0.371	16.04	
4	0.019	42	0.583	11.31	
5	0.038	65	0.512	12.98	
6	0.031	56	0.459	12.13	17.21
7	0.014	71	0.547	21.42	28.78
8	0.026	80	0.424	19.98	
9	0.024	75	0.463	16.55	21.77
10	0.011	80	0.415	21.21	
11	0.017	77	0.439	20.97	12.71
12	0.016	77	0.507	21.23	3.15
13	0.017	77	0.473	21.10	
14	0.010	74	0.523	22.00	1.78
15	0.010	71	0.572	22.02	
16	0.010	68	0.600	21.85	
17	0.014	70	0.600	21.34	
18	0.014	72	0.558	21.23	1.20

selected as the equation to relate the response *R* to the flow-rate and pH of the mobile phase and to the temperature of the column parameters:

$$VCP = axyz + bx^2 + cy^2 + dz^2 + exy^2 + fxz^2 + gy^2z^2 + hx + iy + jz + k$$

where *a, b, c, ..., j* are the coefficients to be evaluated, *x* is the concentration of the sulphuric acid, *y* is the

temperature of the column and *z* is the flow-rate of the mobile phase.

After the experiments, the values of the different coefficients were as follows: *a* = -4.21, *b* = -1831.66, *c* = -0.01, *d* = 224.20, *e* = .70, *f* = 201.38, *g* = -0.10, *h* = 76.02, *i* = 1.21, *j* = -183.55 and *k* = 11.62. These parameters were calculated by a simplex method and the value to be minimized is the sum of the residual square between the calculated values and the theoretical values, $\Sigma(R_{\text{calc}} - R_{\text{theo}})^2$. The results were obtained with a $\Sigma(R_{\text{calc}} - R_{\text{theo}})^2 = 19.55$, an acceptable value for this method with the number of experiments carried out. The different values obtained are given in Table II.

To obtain a graphic representation of the response of the variables to be optimized, it was necessary to fix one variable and to represent the response of the other two. In Fig. 4 the representation of the response of the temperature and concentration of the sulphuric acid on the mobile phase at a constant flow-rate of 0.6 ml/min can be seen. Fig. 4 shows that the temperature is a more impor-

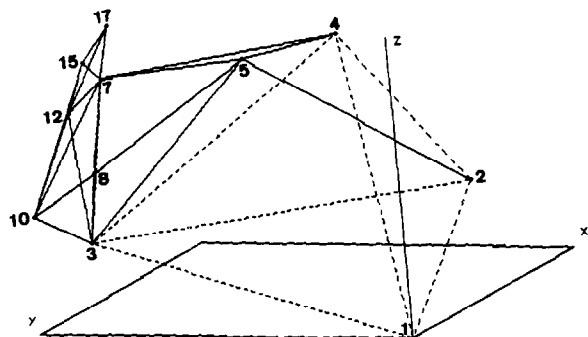


Fig. 1. Movements of the simplex method when the *VCP* criterion is used.

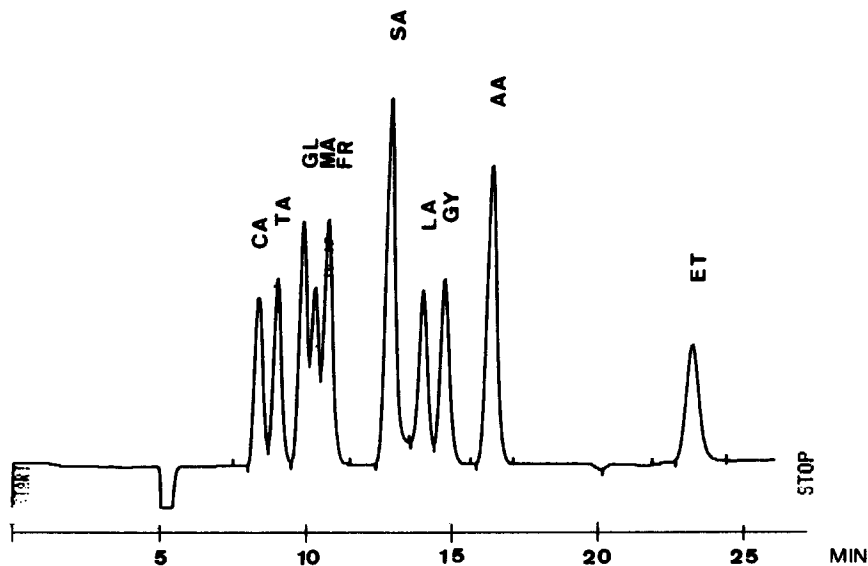


Fig. 2. Chromatogram of a standard solution under the optimum conditions chosen with the simplex method when the *VCP* criterion is used. Temperature, 71°C; flow-rate, 0.572 ml/min; concentration of sulphuric acid in the mobile phase, 0.01 *N*. CA = citric acid; TA = tartaric acid; GL = glucose; MA = malic acid; FR = fructose; SA = succinic acid; LA = lactic acid; GY = glycerol; AA = acetic acid; ET = ethanol.

tant variable than the concentration of sulphuric acid in the mobile phase, and the maximum response was obtained at a low concentration (with a low importance of this variable in this region) and a high value of the temperature, near 70°C.

Fig. 5 shows the variation of the response surface in front of the temperature and the flow-rate at a concentration constant of 0.026 *N*. In this instance, a major importance of the variable temperature compared with the variable flow-rate is observed. The

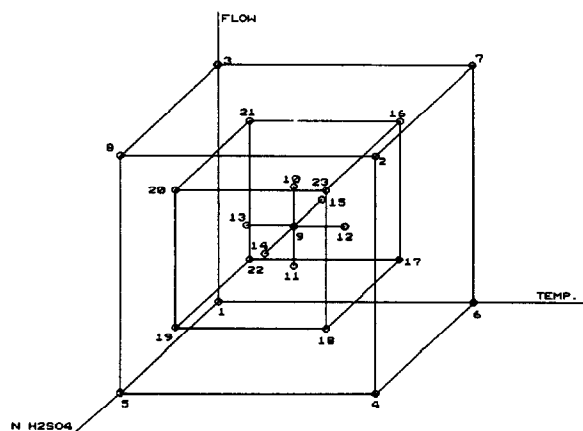


Fig. 3. Experimental design used in the modelling response surface method.

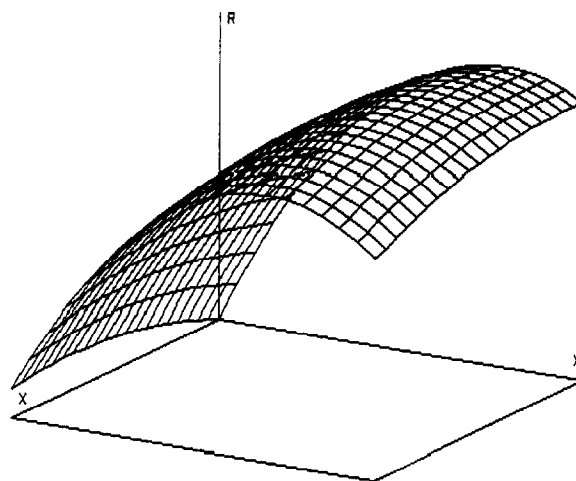


Fig. 4. Graph of response vs. temperature (*Y*) and sulphuric acid concentration (*X*) in the mobile phase at a flow-rate of 0.572 ml/min.

TABLE II
EXPERIMENTAL POINTS USED WITH THE MODELLING RESPONSE SURFACE METHOD

Expt. No.	Variable			R_{theo}	R_{calc}	$R_{\text{calc}} - R_{\text{theo}}$
	H ₂ SO ₄ concentration (N)	Temperature (°C)	Flow-rate (ml/min)			
1	0.010	30	0.300	5.15	5.87	-0.72
2	0.050	80	0.600	21.18	19.92	1.26
3	0.010	30	0.600	11.37	10.64	0.73
4	0.050	80	0.300	17.96	18.93	-0.97
5	0.050	30	0.300	7.57	6.26	1.31
6	0.010	80	0.300	19.56	19.65	-0.09
7	0.010	80	0.600	20.86	22.65	-1.40
8	0.050	30	0.600	10.69	11.94	-1.24
9	0.030	55	0.450	15.47	15.25	0.23
10	0.030	55	0.525	16.45	17.39	-0.94
11	0.030	55	0.375	14.04	15.63	-1.60
12	0.030	68	0.450	16.17	16.85	-0.38
13	0.030	42	0.450	11.65	11.09	0.56
14	0.040	55	0.450	9.68	10.06	-0.38
15	0.020	55	0.450	17.96	18.74	-0.77
16	0.018	70	0.540	20.78	19.57	1.20
17	0.018	70	0.360	12.98	13.01	-0.02
18	0.042	70	0.360	18.30	17.45	0.85
19	0.042	40	0.360	13.15	12.72	0.44
20	0.042	40	0.540	19.23	17.88	1.35
21	0.018	40	0.540	9.42	10.21	-0.79
22	0.018	40	0.360	16.18	15.15	1.03
23	0.042	70	0.540	15.42	14.98	0.44
$\Sigma(R_{\text{calc}} - R_{\text{theo}})^2 =$						19.55

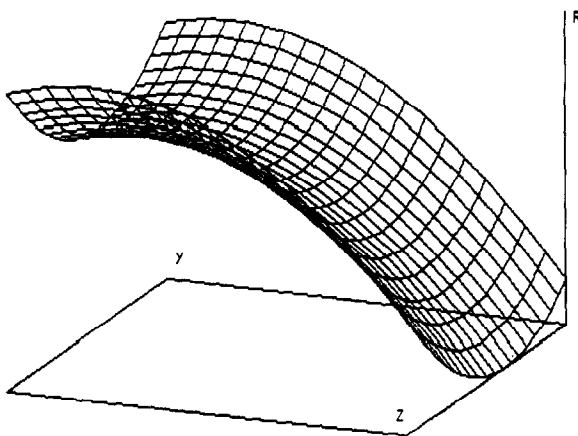


Fig. 5. Graph of response vs. temperature (Y) and flow-rate (Z) at a sulphuric acid concentration of 0.026 N.

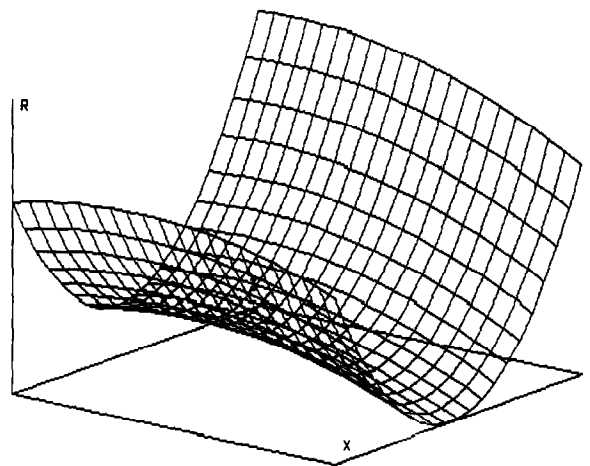


Fig. 6. Graph of the response vs. flow-rate (Z) and sulphuric acid concentration in the mobile phase (X) at a temperature of 74°C.

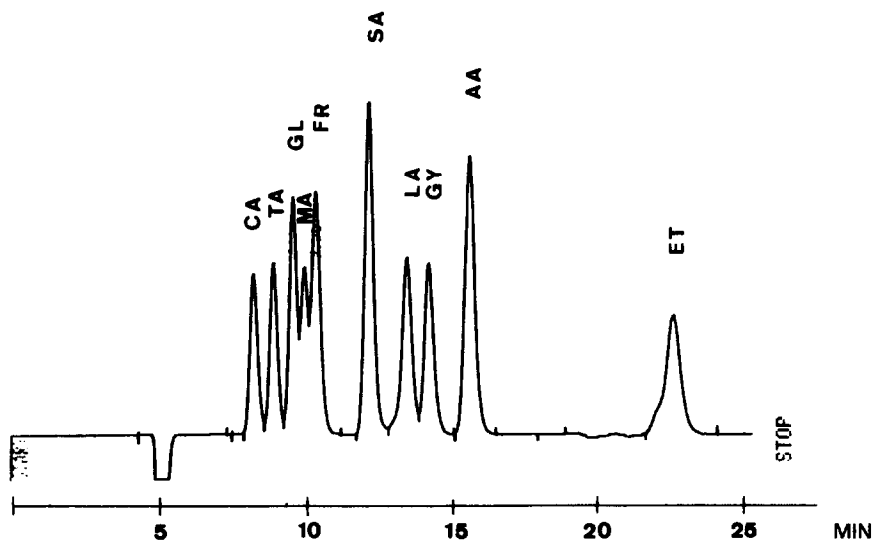


Fig. 7. Chromatogram of a standard solution under the optimum conditions. Temperature, 74°C; flow-rate, 0.6 ml/min; sulphuric acid concentration in the mobile phase, 0.026 *N*. Abbreviations as in Fig. 2.

best results were obtained at high temperature and extreme values of the parameter space for the flow-rate.

In Fig. 6, the influence of the flow-rate and concentration of sulphuric acid at constant tempera-

ture on the response can be observed. Similar results were obtained to those in the Fig. 5, the best results at a low or a high flow-rate and a small influence of the concentration of the sulphuric acid parameter, better at low concentrations. Although good results

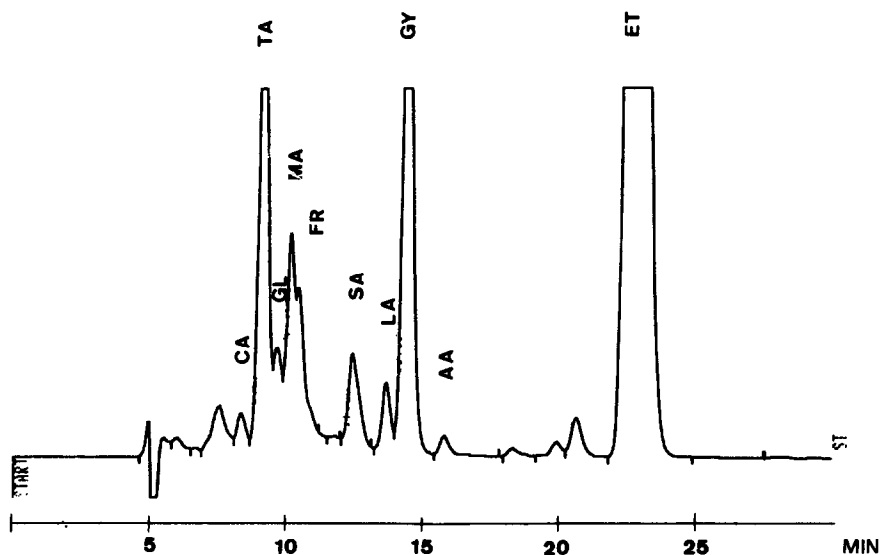


Fig. 8. Chromatogram of a dry white wine under the optimum conditions. Abbreviations as in Fig. 2.

are obtained at low and high values of flow-rate, the best response corresponds to high values of the flow-rate, as in the Fig. 5.

The optimum values of the parameters were a temperature near 70°C, a high flow-rate and a low concentration of sulphuric acid in the mobile phase. The optimum conditions considered were temperature 74°C, flow-rate 0.600 ml/min and concentration of sulphuric acid 0.026 *N*. The chromatogram obtained with these conditions is shown in Fig. 7. The results obtained with the simplex method and the modelling response surface method were similar and no important differences can be observed between the two chromatograms despite the different sulphuric acid concentrations, mainly owing to the small influence of this parameter.

The above chromatographic conditions were applied to determine these compounds in a white wine and the results obtained are shown in Fig. 8.

CONCLUSIONS

The simplex method and the modelling response surface method were used to optimize the different variables in an ion-exchange process. A modification of the *CRF* criterion was used as the parameter to be maximized in the separation of the carboxylic acids, sugars, glycerol and ethanol in wine samples.

The *VCP* criterion can be applied when highly different resolution values are found between the various peak pairs in the chromatographic separation. This criterion increases the importance of the badly resolved peaks in resolution terms.

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