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Examination of a new chromatographic function, based on an exponential resolution term, for use in optimization strategies: application to capillary gas chromatography separation of phenols

Vicki M. Morris, Jeff G. Hughes, Philip J. Marriott*

Department of Applied Chemistry, Royal Melbourne Institute of Technology, GPO Box 2476V, Melbourne 3001, Australia

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Abstract

An objective function has been developed to express the quality of a chromatogram with regard to resolution and time. The function is used as the response variable in an optimization strategy involving a central composite experimental design, multi-linear regression and response surface modelling. This function is compared with an existing objective function in an optimization strategy for the separation of phenols. The capillary gas chromatography settings for column head pressure, initial oven temperature and temperature program rate for the optimum separation are found using the strategy. A surprisingly high temperature rate (22°C/min) was predicted for the optimum separation conditions.

Keywords: Optimization; Chromatographic exponential function; Phenols

1. Introduction

Objective functions (OF) are functions designed to give a single response value that reflects the quality of a chromatogram. This value can be used in optimization strategies that involve analyses such as multi-linear regression. Guillaume et al. [1] used a 'desirability function' along with a central composite design (CCD) and simplex optimization to investigate the effects of gas flow-rate and column head pressure on the separation of eight *p*-hydroxybenzoic esters by gas chromatography (GC). Olsson and Kaufmann [2] used the chromatographic resolution statistic (CRS) as the response for a full factorial design to optimize separation for gas liquid chromatography. Wenclawiak and Hees [3] used the Morgan and Deming version of the chromatographic response function (CRF) along with window diagrams for the optimization of the separation of polyaromatic hydrocarbons by using high-performance liquid chromatography (HPLC). Alternative approaches to the objective function have been to use multi-criteria decision making (MCDM) as used by Smilde et al. [4,5] along with an overlapping resolution mapping (ORM) type technique to optimize separations using HPLC. Drylab–GC has also been used for optimizing instrument conditions [6,7]

In selecting an appropriate OF to use, the criteria for the optimum chromatogram need to be established. The OF needs to optimize to these criteria when used with multi-linear regression. Care needs to be taken when determining an appropriate OF due to the manner in which it ranks chromatograms as rankings can be very subjective. Even when ex-

*Corresponding author

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perienced chromatographers rank a series of chromatograms there may be significant differences in their rankings. It is easy enough to rank the chromatograms with all peaks resolved within a minimum time, but the process becomes difficult when there are several different peak pairs with varying degrees of resolution and different total elution times. Typical considerations that define chromatogram quality include resolutions of peak pairs, total analysis time and the uniformity of peak separation. With respect to these considerations an OF can be constructed which compares chromatograms quantitatively. Experimental conditions that affect chromatographic quality can then be studied with a view to optimizing the objective function.

Objective functions that consider both the time and the resolution have two competing aims – to maximize resolution but to minimize run time. The relative weighting of these two parameters in the function is an important factor in considering an objective function's suitability. It is also important to understand how the OF approaches the optimum value and also what parameters are used to define the optimum. For example, consider the chromatographic response function (CRF, Eq. (1)), introduced by Berridge [8] which optimizes to a maximum. Summing the resolutions results in the unresolved peak pairs having little influence on the function value compared to the pairs with large resolution. This approach results in the quality of the chromatogram being determined by the well resolved peak pairs, where logically it is the poorly resolved pairs that cause analytical problems. Refer to Berridge [9], Cela [10] and Schoenmakers [11] for further discussions on other objective functions.

$$\mathrm{CRF} = \sum_{i=1}^{L} R_i + L^{w_1} - w_2|T_A - T_L| - w_3(T_1 - T_o) \quad (1)$$

where R_i is the resolution of the ith peak pair, L is the number of peak pairs, T_A, T_L, T_1 and T_o are the maximum acceptable time, retention time of final peak, retention time of first peak and the minimum retention time of first peak respectively. w_1 to w_3 are weighting factors selected by the operator, usually set at between 0–3.

The chromatographic resolution statistic (CRS, Eq. (2)) was developed by Schlabach and Excoffier [12]. Olsson and Kaufmann [2] used the CRS in a factorial design for optimizing gas–liquid chromatography conditions. This function can be broken into three parts. The first term optimizes to a minimum of zero when the resolution is optimal (usually $R_{opt} = 1.5$). If the resolution is zero or equal to the minimum resolution then this term is undefined as singularities exist in the function at these points. The second term is a variation of the relative resolution product, RRP [13], and gives a value of 1 for uniform separations. It usually fluctuates between values of 1 and 2. The final part deals with the time of the run. This function gives more weight to time in relation to resolution, which can result in a chromatogram of shorter run time but with unresolved peaks being favoured over a well resolved chromatogram that has a longer run time.

$$\mathrm{CRS} = \left\{ \sum \left[\frac{(R_i - R_{opt})^2}{R_i (R_i - R_{min})^2} \right] + \sum \frac{(R_i)^2}{a\bar{R}^2} \right\} \frac{T_f}{n} \quad (2)$$

R_i, $\bar{R}$, R_{opt}, and R_{min} are the resolution for the ith peak pair, the average resolution, the desired optimum resolution and the minimum acceptable resolution respectively. T_f is the elution time of the final peak, a is the number of resolution elements and n is the number of peaks. The summation is over all peaks, $i = 1$ to $n - 1$ (the original reference does not specify summation range).

In order to satisfy the objective function requirements we have developed a new function, which we have called the chromatographic exponential function (CEF). This function allows a choice of the emphasis between time and resolution by introduction of an adjustable parameter. In this study we have used both the CEF and CRS as the chromatogram response in the optimization of the capillary gas chromatography separation of a 17 component phenol mixture PHM-804 using a central composite experimental design [14] and multi-linear regression, with the CRS chosen as a comparison to the CEF.

2. Experimental

2.1. Instrumentation

A Shimadzu GC14A gas chromatograph (Kyoto, Japan) with a flame ionization detector and AOC-17

auto injector was used. The GC was connected to an IBM compatible computer using a CBM-101 communication bus module; Shimadzu Class-GC10 software was used to control the temperature, the auto injector and for data acquisition.

2.2. Chromatography

A BPX5 (SGE International, Ringwood, Australia) non-polar, 5% phenyl equivalent modified siloxane capillary column (12 m×0.22 mm I.D.) with 0.25 μm film thickness was used. Conditions were injection temperature 220°C, detector temperature 250°C, split ratio 1:25, purge ~10 ml/min and high purity helium (Linde gas, Melbourne, Australia) carrier gas. Initial oven temperature, temperature program rate and column head pressure were adjusted according to the experimental design as in Table 2. Each chromatographic run had a 1 min isothermal hold time at the initial oven temperature before onset of temperature programs.

2.3. Sample

The Ultra Scientific phenols mix PHM-804 was obtained from Alltech (Melbourne, Australia) Lot no. H-1534. This sample mixture contains 17 components in methanol.

2.4. Software

The generation of the central composite designs and the multi-linear regression analysis were performed on Minitab for Windows version 10 (Minitab, State College, PA, USA). Statistica version 4.5 (Statsoft, Hamburg, Germany) was used to graph response surfaces. Optima were located by canonical analysis ([14], p. 332) using Mathcad version 5.0 (Mathsoft, MA, USA).

3. Results and discussion

Our criteria for an optimum chromatogram are to have all peaks resolved to at least 1.5 and all components eluted within a minimum time, although we are willing to accept a chromatogram with slightly less resolution rather than a chromatogram that elutes at a time greater than our stipulated maximum time. In order for the objective function to approach this optimal chromatogram it needs the following characteristics:

1. The emphasis on time should be minimal unless the maximum acceptable time is exceeded.
2. Excessive resolution of any peak pair greater than the desired optimal resolution should have minimal contribution to chromatogram quality. The significant contributions to chromatogram quality should come from the unresolved peaks, with decreasing contribution as the optimum resolution is reached.

Both the CEF and the CRS optimize to a minimum value. The approach the CEF (Eq. (3)) takes to resolution is different to the CRS, since the CEF does not have any singularities in the resolution factor (Fig. 1). The time domain of the CEF for final peak elution is also different. An expanded view of the functions is shown in Fig. 2. Both functions reach a minimum at the set optimum resolution, $R_s = 1.5$, and, while they differ in magnitude at $R_s > 1.5$, qualitatively give similar results. Adjusting a will alter the slope of the resolution factor of the CEF (Fig. 3). Decreasing a means a lower slope and therefore a decrease in the significance of resolution as against time. Fig. 3 also shows the change in the

Table 1
Resolution and respective objective function values for a series of simulated chromatograms shown in Fig. 5

Chromatogram	Resolution									t_1 (min)	CEFa	CEFb	CRS
	1,2	2,3	3,4	4,5	5,6	6,7	7,8	8,9	9,10				
a	4.59	4.58	7.88	8.74	7.36	0.98	3.48	1.53	4.09	3.83	34.35	28.85	1.255
b	4.59	15.77	9.46	3.57	4.41	1.94	10.22	4.11	2.91	5.25	16.63	14.85	1.230
c	4.59	15.77	9.46	3.57	4.41	1.94	10.21	4.11	4.96	5.50	17.03	15.42	1.259
d	4.59	15.77	9.46	3.57	4.41	1.94	10.21	4.11	8.79	6.00	17.71	16.54	1.327
e	3.59	16.41	12.09	2.79	5.28	2.13	13.01	1.91	1.18	5.68	19.37	17.74	1.436

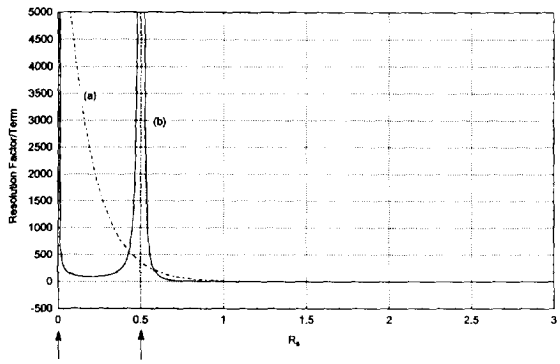

Fig. 1. Comparative plots of the resolution domain for the resolution terms/factors of the (a) CEF and (b) CRS objective functions according to the expressions $(1-e^{3(R_{opt}-R_i)})^2$ and $(R_i - R_{opt})^2/(R_i(R_i - R_{min})^2)$ respectively, for $R_{opt}=1.5$ and $R_{min}=0.5$. The arrows indicate the undefined regions of the CRS where $R_i=R_{min}=0.5$ and $R_i=0$.

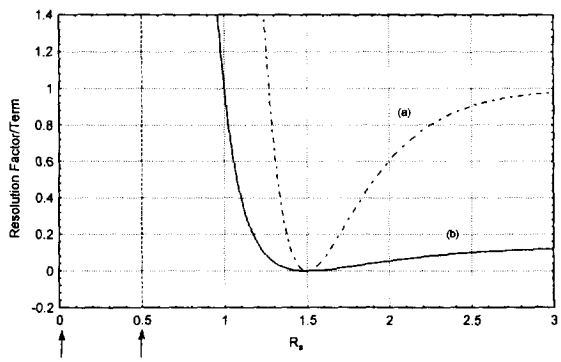

Fig. 2. Expanded region of Fig. 1.

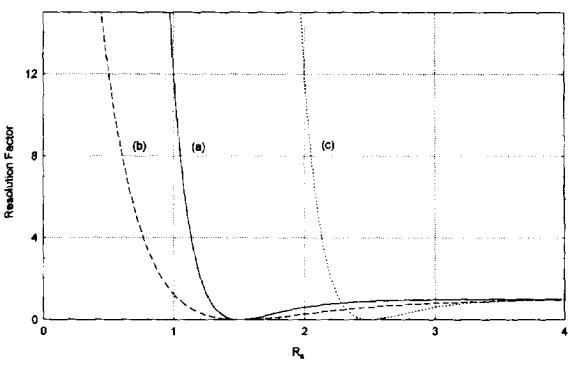

Fig. 3. Plot of the resolution factor over the resolution domain for the CEF objective function, for the following values of a and R_{opt}; (a) $a=3$, $R_{opt}=1.5$, (b) $a=1.5$, $R_{opt}=1.5$ and (c) $a=3$, $R_{opt}=2.5$.

function when the optimum resolution chosen for the function is altered. The addition of 1 to the resolution factor ensures that when the resolutions are all at an optimum the CEF can still differentiate between the times of the chromatograms, otherwise time would not have an effect in this case.

$$\mathrm{CEF}=\left[\left(\sum_{i=1}^{n-1}\left(1-e^{a(R_{opt}-R_i)}\right)^2\right)+1\right]\left[1+\frac{t_f}{t_{max}}\right] \quad (3)$$

R_{opt} and R_i are selected optimum resolution and the resolution for the ith peak pair respectively, t_{max} and t_f are the maximum acceptable time and the elution time of the final peak respectively, a is the slope adjustment factor and n is the number of expected peaks. The resolution factor is given in the first square brackets and the time factor in the second square brackets.

Fig. 4 shows the relationship of the time factor to final peak times. The time factor varies between 1 and 2 provided the maximum time is not exceeded. Increasing t_{max} will decrease the effect time has on the function, whilst using $(t_f/t_{max})^2$ instead of (t_f/t_{max}) will increase the effect time has after the maximum time has been exceeded by causing this factor to increase more rapidly.

There is no need to identify the peaks or to be concerned with peak cross-overs when using either the CEF or the CRS as they will not affect the response value given by these two functions. Peak

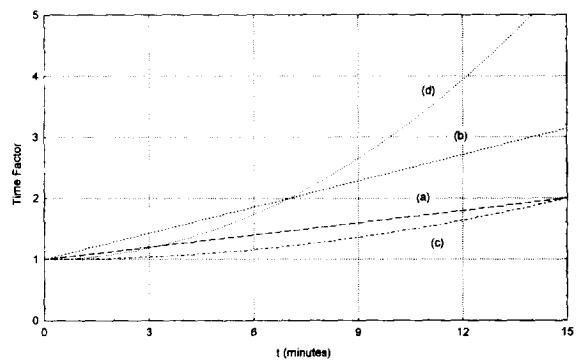

Fig. 4. Plots of the time factors of the CEF over the time domain for the following combinations; (a) $1+(t_f/t_{max})$, $t_{max}=15$, (b) $1+(t_f/t_{max})$, $t_{max}=7$, (c) $1+(t_f/t_{max})^2$ $t_{max}=15$ and (d) $1+(t_f/t_{max})^2$, $t_{max}=7$.

cross-overs will possibly result in the actual response surface having local and global optima. Further investigation is required and is currently in progress to determine the effect of peak cross-overs on the modelled response surface and to ensure that the global optimum is found.

In order to test the sensitivity of CRS and CEF towards time and resolution differences, a series of simulated chromatograms was used. The simulated chromatograms in Fig. 5 have their resolution, final peak elution time and the values computed for CRS, CEFa and CEFb tabulated in Table 1. CEFa and CEFb incorporate time terms (t_f/t_{max}) and $(t_f/t_{max})^2$ respectively. The value of a used was 3 and selected variables were; $t_{max}=7$ min, $R_{opt}=1.5$ and $R_{min}=0.5$. The difference between chromatogram 5b, 5c and 5d is the elution time of the final peak (leading to a higher resolution between the final two peaks). This difference is reflected in the values given by the three OFs, which all rank 5d worse than 5b and 5c (i.e. giving a larger value for the OF). Due to the significant emphasis that it places on time, chromatogram 5a with a poorly resolved peak pair of $R_s=0.98$ and an overall elution time of 3.83 min is considered by the CRS to be a better quality chromatogram than chromatograms 5c, 5d and 5e. The CEF function considers this chromatogram to be not as good as the other four simulated chromatograms. Both OFs consider the resolution of 1.18 for the final peak pair for chromatogram 5e to be significantly poor enough so as to recognise it as a less desirable chromatogram than 5d which has all peaks resolved but a higher final peak elution time of 6 min as opposed to 5.68 min for 5e. As discussed previously, by adjusting the value of a and t_{max}, the emphasis on resolution compared to time can be altered depending on our preference. For the phenol study a was set at 3 and the maximum acceptable time was chosen to be 15 min.

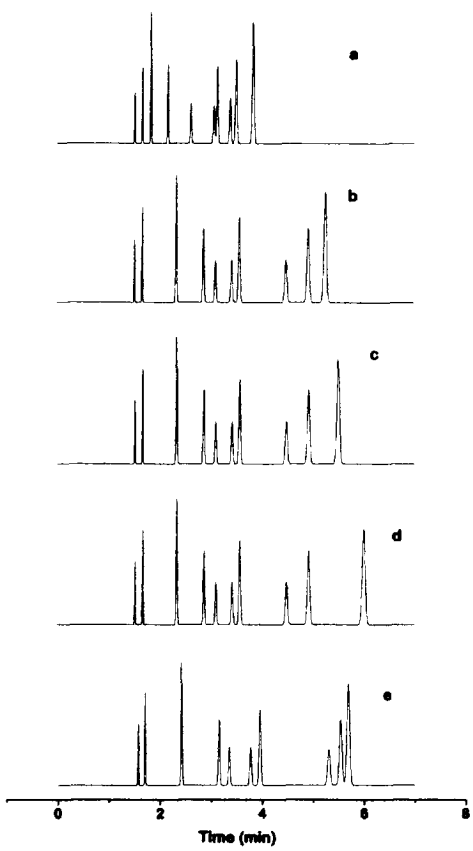

Fig. 5. Series of simulated chromatograms (generated in Microsoft Excel version 4) used to demonstrate the performance of CRS, CEFa and CEFb objective functions. Refer to Table 1 for further information on these chromatograms.

The above functions were used in the study of the optimization of the capillary GC separation of a phenol mixture. A central composite experimental design [14] with 6 centre points, incorporating column head pressure, initial oven temperature and temperature program rate as the design variables was chosen for the optimization. The value of α which ensures rotatability was 1.682. The runs were carried out over two days, hence requiring the experimental design to be orthogonally blocked. Block 1 is the factorial part and block 2 the star part of the design. The order of runs within the blocks were randomised. Table 2 gives the conditions for each run and Table 3 presents the experimental results of these runs. The 3-methylphenol and 4-methylphenol co-eluted for all 20 runs. These two components were treated as one for the calculations of the OFs.

Fig. 6 is the CEFb response model for the initial oven temperature and temperature program rate when the column head pressure is held at 1 kg/cm^2. The relative standard deviations for the replicated centre points were 0.4% for CRS and 1.2% for the CEFa and CEFb. The optimum conditions are located where the surface is at a minimum. The initial

Table 2
Central composite experimental design

Run	Column head pressure (kg/cm^2)	Initial oven temperature (°C)	Temperature program rate (°C/min)	Block
1	0.82	60.0	17.0	1
2	0.94	48.0	24.7	1
3	0.69	72.0	9.3	1
4	0.82	60.0	17.0	1
5	0.94	72.0	9.3	1
6	0.69	48.0	24.7	1
7	0.82	60.0	17.0	1
8	0.82	60.0	17.0	1
9	0.94	48.0	9.3	1
10	0.69	48.0	9.3	1
11	0.69	72.0	24.7	1
12	0.94	72.0	24.7	1
13	0.61	60.0	17.0	2
14	0.82	39.8	17.0	2
15	0.82	80.2	17.0	2
16	0.82	60.0	4.1	2
17	0.82	60.0	17.0	2
18	0.82	60.0	30.0	2
19	1.0	60.0	17.0	2
20	0.82	60.0	17.0	2

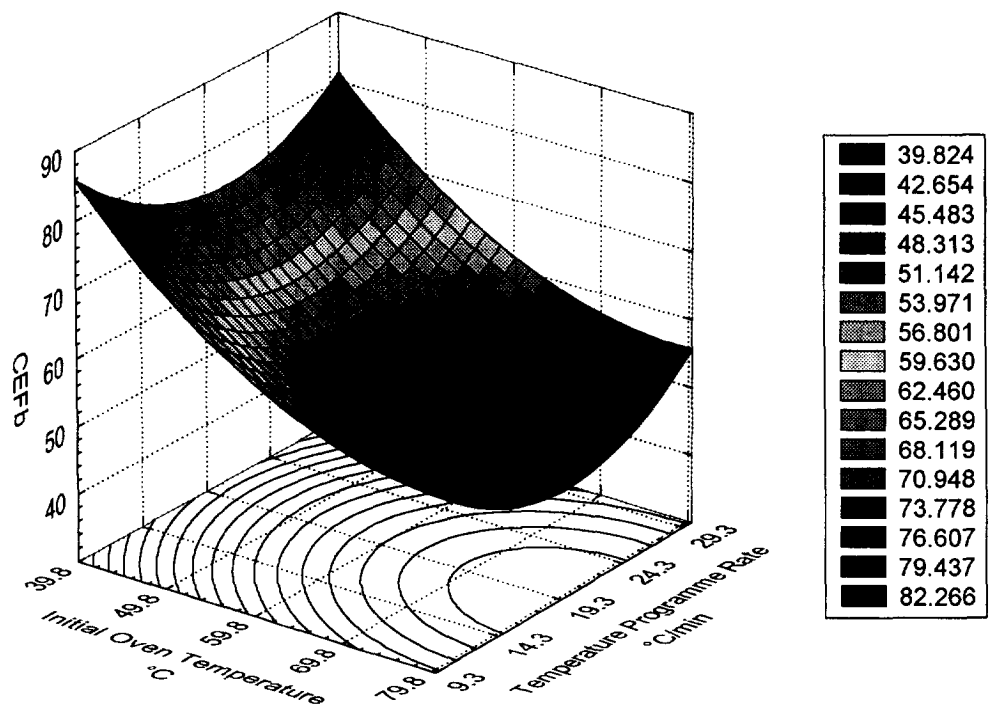

Fig. 6. Response model for the experimental design presented in Table 2 and based upon the CEFb objective function for chromatogram response. The column head pressure is held at a 1 kg/cm^2.

Table 3
Resolution and respective objective function values for the 20 chromatographic runs shown in Table 2 for the phenol mixture

Run number	Tf	1,2	2,3	3,4	4,5	5,6	6,7	7,8	8,9	9,10	10,11	11,12	12,13	13,14	14,15	15,16	16,17	CRS	CEFa	CEFb
1	9.57	15.89	3.65	18.84	5.84	14.86	2.45	6.43	9.34	21.88	15.35	1.08	31.82	2.95	4.09	11.99	22.42	1.803	36.68	31.51
2	7.81	10.39	2.86	15.75	4.90	12.36	1.81	5.54	7.62	17.21	12.89	0.99	26.35	2.33	4.09	10.29	21.36	1.729	42.24	35.30
3	13.83	26.78	4.34	18.67	6.28	17.14	2.98	6.96	10.27	24.85	17.08	1.10	33.87	3.76	3.24	13.60	24.79	2.511	41.28	39.73
4	9.49	16.35	3.53	18.87	5.88	14.84	2.52	6.50	9.55	22.20	15.57	1.07	30.38	3.03	3.88	11.90	22.46	1.790	36.98	31.71
5	12.90	22.46	4.20	20.30	6.48	16.91	3.72	6.97	11.08	27.74	18.19	1.21	40.10	5.06	2.77	15.56	28.15	2.138	33.42	31.26
6	8.49	9.64	3.28	13.98	4.29	11.57	1.16	5.54	6.63	15.39	12.28	0.92	23.95	1.76	3.95	8.79	19.47	2.455	60.88	51.33
7	9.54	15.99	3.60	18.65	5.80	14.81	2.46	6.43	9.53	21.93	15.50	1.08	32.70	3.21	3.89	11.92	22.57	1.796	36.33	31.19
8	9.55	16.21	3.60	18.83	5.83	14.94	2.51	6.53	9.66	22.06	15.57	1.08	32.57	3.16	3.78	12.11	22.59	1.785	36.07	30.97
9	15.46	13.93	2.00	22.25	6.08	13.22	4.32	5.53	10.78	27.82	16.31	1.13	42.45	5.74	2.65	15.62	28.50	2.788	40.18	40.80
10	16.39	13.11	2.46	20.59	5.71	14.51	4.20	6.64	11.00	26.40	16.56	1.13	38.13	4.65	3.31	14.17	25.82	2.939	41.74	43.76
11	7.54	22.77	4.15	14.24	5.05	14.90	1.06	6.02	7.37	16.20	13.24	0.95	24.07	1.69	3.97	8.63	18.73	2.058	57.85	48.22
12	6.83	20.22	4.13	16.00	5.39	15.13	1.72	6.22	8.48	19.16	14.58	1.05	28.19	2.40	3.96	10.30	20.86	1.312	34.06	28.25
13	10.33	15.80	3.78	16.42	5.30	13.93	1.62	6.10	8.14	18.34	13.91	1.01	27.45	2.27	3.92	9.67	19.30	2.172	44.79	39.11
14	10.68	8.95	2.22	18.14	5.78	14.07	2.84	6.16	9.52	21.59	14.69	1.07	35.83	3.72	3.89	12.16	22.97	2.104	38.58	33.96
15	8.32	23.40	4.33	15.24	5.59	16.41	1.88	6.72	8.98	21.18	15.92	1.07	28.42	2.63	3.73	11.45	21.50	1.531	34.42	28.95
16	26.30	19.86	2.81	25.32	6.93	15.16	6.05	6.13	12.88	34.26	18.40	1.20	50.90	8.25	0.70	19.55	32.64	39.23	322.3	476.9
17	9.53	15.77	3.51	18.53	5.75	14.62	2.43	6.29	9.41	21.81	15.34	1.08	32.87	3.35	3.95	12.31	22.73	1.802	36.47	31.30
18	6.80	13.57	3.51	14.89	4.75	12.99	1.25	5.72	7.18	15.59	12.29	0.96	25.81	1.94	4.09	9.03	20.10	1.653	46.23	38.34
19	9.09	15.03	3.10	19.40	5.95	14.17	2.98	6.29	9.96	23.03	15.20	1.09	34.44	3.81	3.53	12.97	25.10	1.705	34.81	29.63
20	9.51	16.09	3.50	18.41	5.77	14.70	2.49	6.40	9.58	21.94	15.17	1.09	34.57	3.53	3.93	12.06	22.98	1.792	35.85	30.76

Note: Peak 1 refers to the solvent peak.

oven temperature can be seen to be optimizing at conditions outside the region of the response model as occurs also for the column head pressure. For instance, in the experimental range chosen a minimum CEFb value is found for an initial oven temperature of ~80°C, but since the CEFb response is still decreasing, it suggests an even higher initial oven temperature may give a better result. Initially, 80°C was chosen as the range maximum for initial oven temperature due to the possibility of the first component co-eluting with the solvent peak. A further design needs to be initiated to see if a quicker run time and a higher resolution between peaks 11 and 12 can be established. Another design will not locate any conditions that will separate the 3-methylphenol and 4-methylphenol, only a change of column type will achieve separation of these components. Within the region of the response model the optimum conditions using the CEFb are at a column head pressure of 1 kg/cm^2, initial oven temperature of 80°C and a temperature program rate of 22°C/min which gives the chromatogram in Fig. 7a. These conditions have the critical peak pair, 11 and 12, better resolved (1.44) than any of the experimental runs within one of the three shortest analysis times (6.973). Another observation was the relative retentions for the peaks 13, 14 and 15. The central member of this group, peak 14, can vary in position from almost overlapping peak 13 to almost overlapping peak 15 depending on the conditions used.

In this example the CRS also located an optimum, since there was no chromatogram where a poor resolution and a significantly shorter overall time occurred, unlike that for the simulated chromatogram. The optimum conditions predicted using the CRS, CEFa and CEFb as the chromatogram responses were as follows: they all gave the same column head pressure of 1 kg/cm^2 and initial oven temperature of 80.2°C, but slightly different optimum temperature program rates of 26.6°C/min, 21.2°C/min and 22.0°C/min respectively. These are all surprisingly high program rates. It is interesting that for the work of both Bautz et al. [6] and Sippola et al. [7], the optimising program Drylab–GC predicted high programming rates of 32 and 40°C/min, although these were outside their experimental region of 4 to 12°C/min and 2.5 to 12°C/min respectively. As stated by Sippola et al. [7] it is unlikely that such a high programming rate would normally be considered.

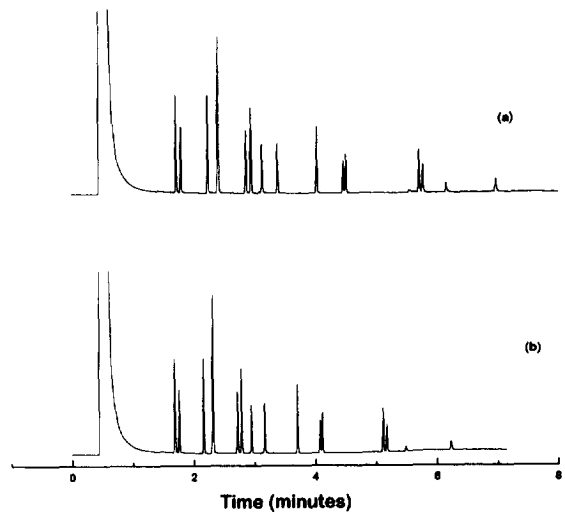

Fig. 7. Chromatograms of phenol mix PHM-804 on a BPX5 capillary column under the following conditions of column head pressure, initial oven temperature and temperature program rates, respectively; (a) 1.0 kg/cm^2, 80°C and 22°C/min and (b) 1.0 kg/cm^2, 80°C and 26°C/min. Components in elution order: phenol, 2-chlorophenol, 2-methylphenol, 4-methylphenol and 3-methylphenol, 2-nitrophenol, 2,4-dimethylphenol, 2,4-dichlorophenol, 2,6-dichlorophenol, 4-chloro-3-methylphenol, 2,4,6-trichlorophenol, 2,4,5-trichlorophenol, 2,4-dinitrophenol, 4-nitrophenol, 2,3,4,6-tetrachlorophenol and 2-methyl-4,6-dinitrophenol and pentachlorophenol.

Figs. 7a and b show the resulting chromatograms for CEFb and CRS which had final elution times of less then 7.0 min, unresolved 3-methylphenol and 4-methylphenol peaks and resolutions for peak pairs 2,4,6-trichlorophenol and 2,4,5-trichlorophenol of 1.45 and 1.36 respectively. The optimum conditions obtained were significantly different from the conditions given in the manufacturer's brochure on the BPX5 25 metre column [15] and the results obtained by Bautz et al. [6] on the DB-5 30 metre column. They resolved the 11 components in the EPA phenol mixture with a final elution time of approximately 22 and 15 min respectively. These 11 components were also readily resolved in our study. The conditions given by SGE were initial temperature 40°C, hold for 1 min, temperature program rate 8°C/min, carrier gas (H_2) pressure of 12 psi (0.84 kg/cm^2).

4. Conclusion

The newly proposed objective function, CEF, is used as the response variable for the optimization of separation and elution time in chromatography. This function has so far achieved similar results to the CRS. However, the CEF has the advantage of not having undefined points and also has less emphasis on elution time over resolution. The simulated chromatogram study indicated that the CRS could place more emphasis on time rather than resolution, and so lead to an incompletely resolved chromatogram being favoured over a fully resolved chromatogram with a longer total elution time. This situation did not arise in the phenol study.

These objective functions along with a central composite design were applied to optimize the capillary GC separation of a phenol mixture. The optimum settings were 1 kg/cm^2 for column head pressure, 80°C for initial oven temperature and a surprisingly high temperature program rate of 22°C/min on a 5% phenyl equivalent modified siloxane column.

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