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Computer simulation for optimization of high-performance liquid chromatography of some phenolic pollutants

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ABSTRACT

Computer simulation was used to optimize high-performance liquid chromatography of phenol and its chloro and nitro derivatives. On the basis of two linear gradient runs of different steepness (RP-18–water + methanol + 1% acetic acid), several simulated gradient runs allowed the optimum gradient programme and flow-rate to be chosen so that the time of analysis could be considerably shortened. Good agreement between simulated and experimental chromatograms was obtained in spite of changes in experimental conditions.

INTRODUCTION

The increasing application of computers in the optimization of chromatographic systems, including gradient elution, is reflected by the recent publication of several books [1–3] and special issues of chromatographic journals [4,5]. The well developed theory of gradient elution [6–8] permits the prediction of the relationships between resolution (R_s) and the gradient programme and other experimental variables.

Advanced computer programs for the simulation and optimization of high-performance liquid chromatography (HPLC) separations have recently been reported by Snyder and co-workers [9,10]: these programs, Drylab I (isocratic) and Drylab G (gradient), analyse the resolution of all pairs of components for various eluent compositions (Drylab I) or gradient programs (Drylab G) on the basis of a few experimental data, *i.e.*, capacity factors (k') for two eluent compositions or two linear gradient runs of various steepness. After the choice of the optimum conditions (good resolution in the shortest possible time), the simulated chromatogram is displayed. The experimental variables can then be further adapted (column length, particle size of the packing, flow-rate, gradient profile) and the modified simulated chromatogram checked for optimum separation.

In this study, the conditions for the HPLC of a group of chloro- and nitrophenols, frequently occurring as water and air pollutants, were optimized using the Drylab G expert system.

TABLE I

CHLORO- AND NITROPHENOLS STUDIED

Compound	Code No.	Compound	Code No.
Phenol	1	4-Chloro-3-methylphenol	6
4-Nitrophenol	2	2,4-Dichlorophenol	7
2,4-Dinitrophenol	3	2,4,6-Trichlorophenol	8
2-Nitrophenol	4	Pentachlorophenol	9
2,3-Dimethylphenol	5		

EXPERIMENTAL

An HP-1050 gradient liquid chromatograph (Hewlett-Packard, Palo Alto, CA, U.S.A.) equipped with a 20-μl sample injector (Rheodyne, Cotati, CA, U.S.A.) and a variable-wavelength UV detector (HP 1050) operated at 280 nm was used. The chromatograms were recorded with a Hewlett-Packard Model 3396A reporting integrator. The stainless-steel column, 150 × 4.6 mm I.D., was packed with 10-μm C_{18}-bonded silica gel (based on Si 100, 18% carbon) additionally silanized with hexamethyldisilazane. The column was prepared and kindly supplied by prof. H. Engelhardt's laboratory (Department of Applied Physical Chemistry, University of Saarland, Saarbrücken, F.R.G.) and had an efficiency of 2300 plates, as determined using toluene as the test solute, eluted with methanol–water (60:40) at a flow-rate of 1.0 ml/min. The column dead volume ($V_m = t_0F$, where t_0 is the column dead time and F is the flow-rate), determined using pure water as the sample with methanol–water (50:50) as mobile phase, was $V_m = 1.4$ ml.

The dwell volume of the equipment, $V_D = t_DF$ (where t_D is the dwell time), was determined by running a blank gradient without the column [11]; it was found to be $V_D = 0.45$ ml. The estimated extra-column band broadening was $\sigma_{ext} = 0.020$.

In gradient runs, methanol (E. Merck, Darmstad, F.R.G.) and water (doubly distilled) were used as components of the mobile phase; both solvents contained 1% (v/v) of acetic acid. The sample was a mixture of nine components (see Table I) dissolved in methanol. The volume of sample injected was 10 μl, which corresponded to *ca.* 50 μg of each component.

RESULTS AND DISCUSSION

As indicated in the Drylab G instruction manual [11], two linear gradients of various slopes were run (gradient times, $t_g = 15$ and 45 min, the methanol content being varied in the range 5–100%. The retention times and peak areas are given in Table II and the chromatograms in Fig. 1a and b.

Comparison of these chromatograms indicates that at $t_g = 15$ some drift of the baseline is obtained. For $t_g = 45$ min all component pairs have $R_s > 1.5$ and the stability of the baseline is satisfactory. Comparison of the peaks in Fig. 1a and b shows that the sequence of peaks is the same.

The next step is to introduce the retention data to the computer in accordance with the Drylab G program. The optimization can be carried out assuming the rou-

TABLE II

RETENTION VALUES FOR TWO GRADIENT RUNS

t_R = Retention time. t_g = 15 and 45 min. Note: Table II and Figs. 1c, 2a and 3a are copies of Drylab G displays.

Code No.	Run 1		Run 2: t_R (min)
	t_R (min)	Area	
1	7.58	104 010	10.58
2	9.04	276 487	15.28
3	9.56	441 062	16.92
4	10.26	167 457	18.75
5	11.01	232 109	21.95
6	11.88	177 688	24.97
7	12.37	334 442	26.32
8	13.77	85 657	30.63
9	15.85	127 520	38.25

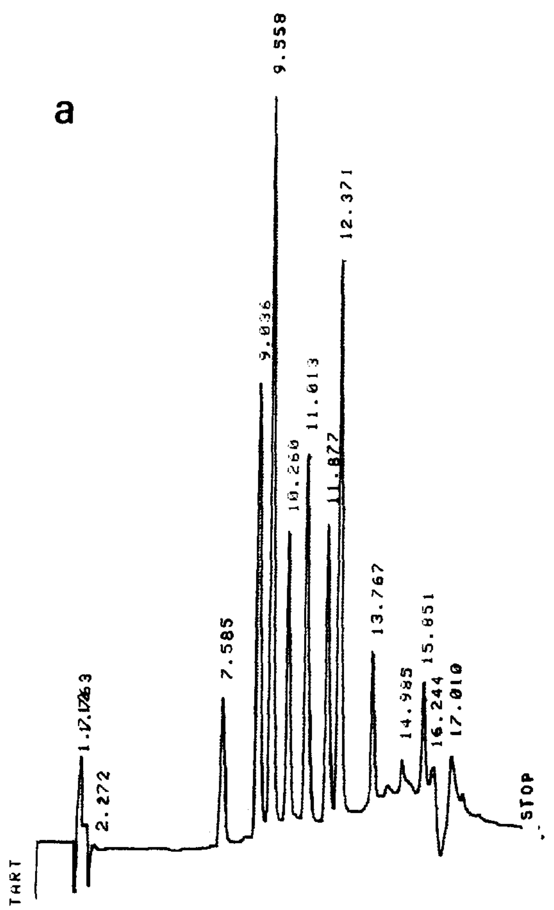

Fig. 1.

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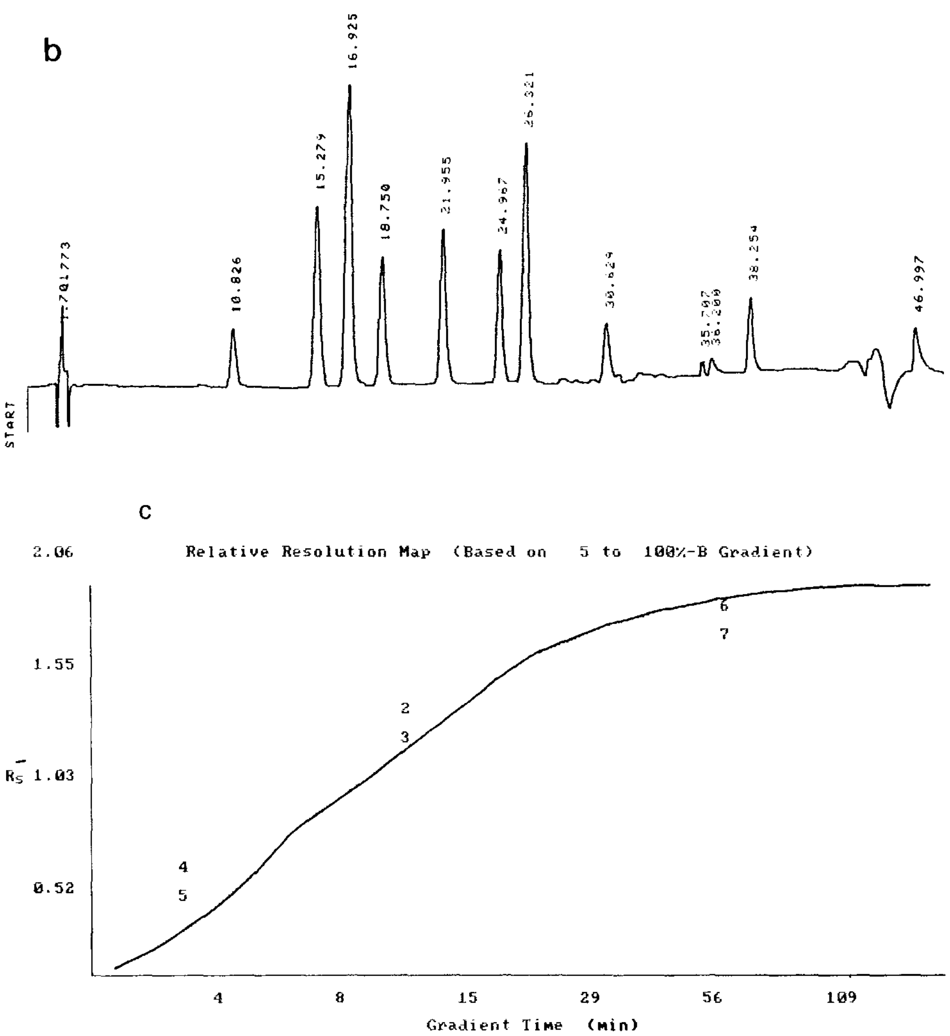

Fig. 1. Separation of chloro- and nitrophenols by gradient elution with 5–100% methanol–water (a) Gradient time t_g = 15 min; (b) t_g = 45 min; (c) R_s *vs.* t_g plot. Values at peaks are retention times in min.

tine efficiency (plate number, $N = 10^4$) or introducing the actual efficiency; in our experiment we chose the latter option ($N = 2302$); the column efficiency was corrected using option 6.6 of the Drylab G manual. The R_s of the critical pair was decreased from 4.023 to 1.93.

The next step consists in the selection of the minimum gradient time for which R_s for the critical pair is above 1.5 and all components are eluted from the column. This step could be done on the basis of the R_s *vs*, t_g relationship [10,11] (Fig. 1c) or from results presented by option 2 in table form (run time, resolution *vs*. gradient time). The optimum t_g was found to be 24 min. For these conditions the retention

TABLE III

RESULTS FOR OPTION 6.5: MULTI-SEGMENTED GRADIENT FOR NINE BANDS IN A 31.0–100.0% MULTI-SEGMENTED GRADIENT RUN OVER 20 min IN TWO SEGMENTS

Gradient: 0 min, 31.0% B; 9 min, 50% B; 20 min, 100% B.

Band No.	$t_{R(calc.)}$	$t_{R(exp.)}$[a]	Band width	Average k'	R_s[b]
1	5.352	5.04	0.435	4.178	3.67
2	7.091	6.95	0.512	5.272	1.65
3	7.963	7.87	0.543	5.722	2.27
4	9.253	9.38	0.594	6.444	3.16
5	11.153	11.58	0.596	6.478	3.57
6	13.078	13.62	0.492	4.987	1.77
7	13.932	14.43	0.475	4.746	5.14
8	16.259	16.61	0.431	4.120	8.60
9	19.619	19.72	0.351	2.993	

[a] Average error in t_R = ±0.29 min (= ±2.4%).

[b] Average error in R_s (difference in t_R) = ±9%.

time of the first peak was too long, 8.96 min (Fig. 1c), so further optimization consisted in finding the maximum initial concentration of methanol at which the earlier obtained separation would be retained. Several simulations, using linear and segmented gradients, permitted the reduction of the retention time of the first peak to 5 min, keeping $R_s > 1.5$ (Table III).

The gradient run simulated for these conditions is shown in Fig. 2a and compared with the experimental run (Fig. 2b, with a blank run for the water–methanol system, Fig. 2c). A comparison of the simulated and experimental chromatograms

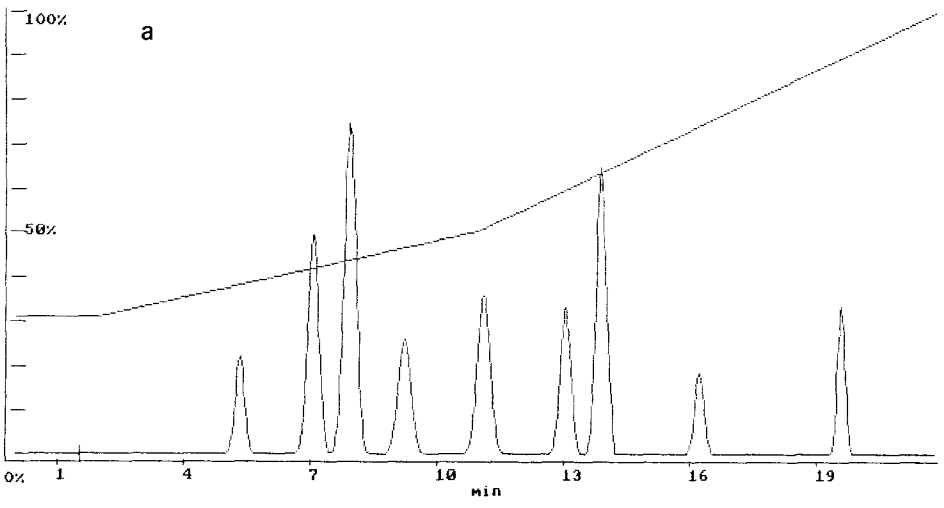

Fig. 2.

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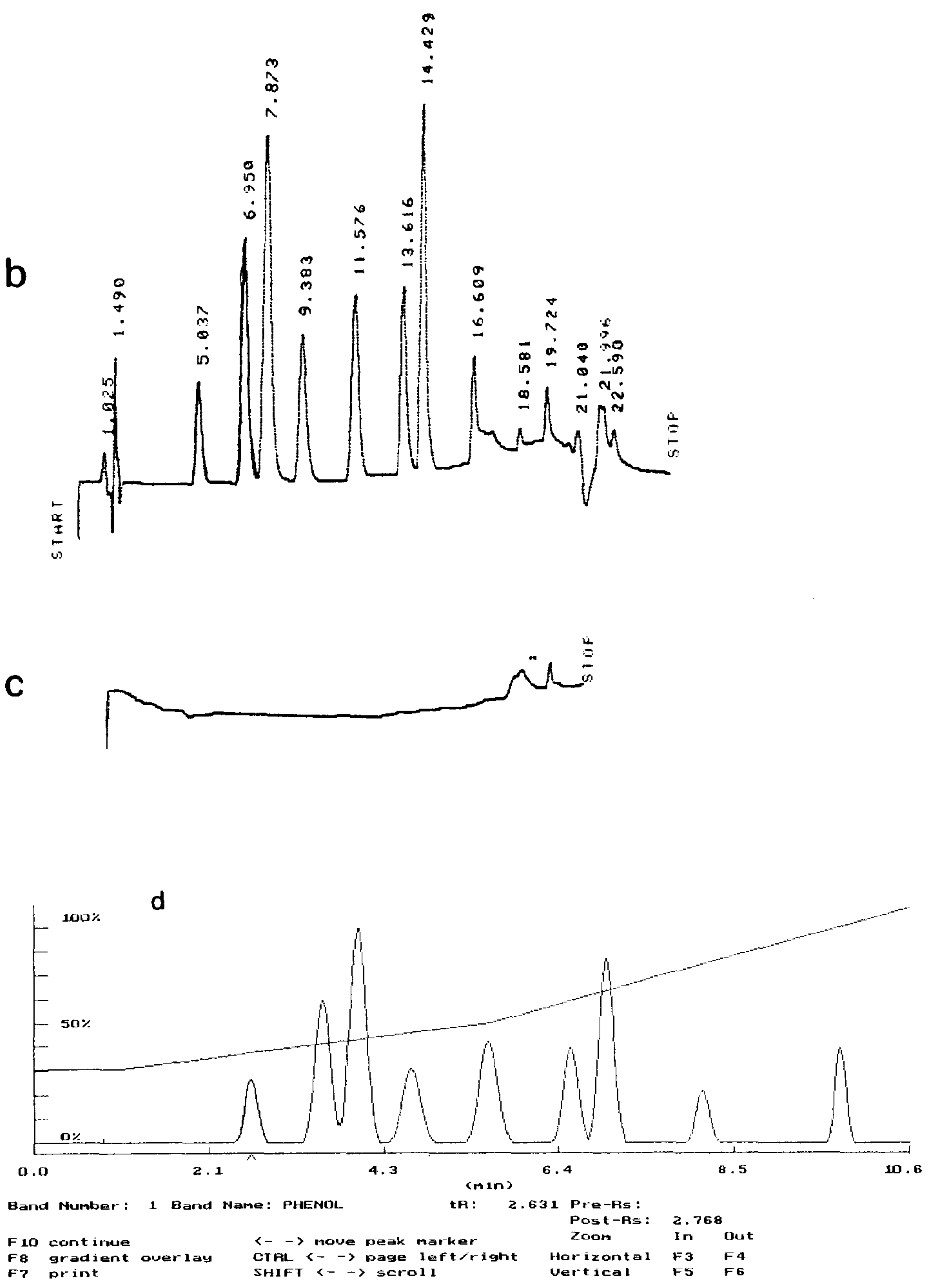
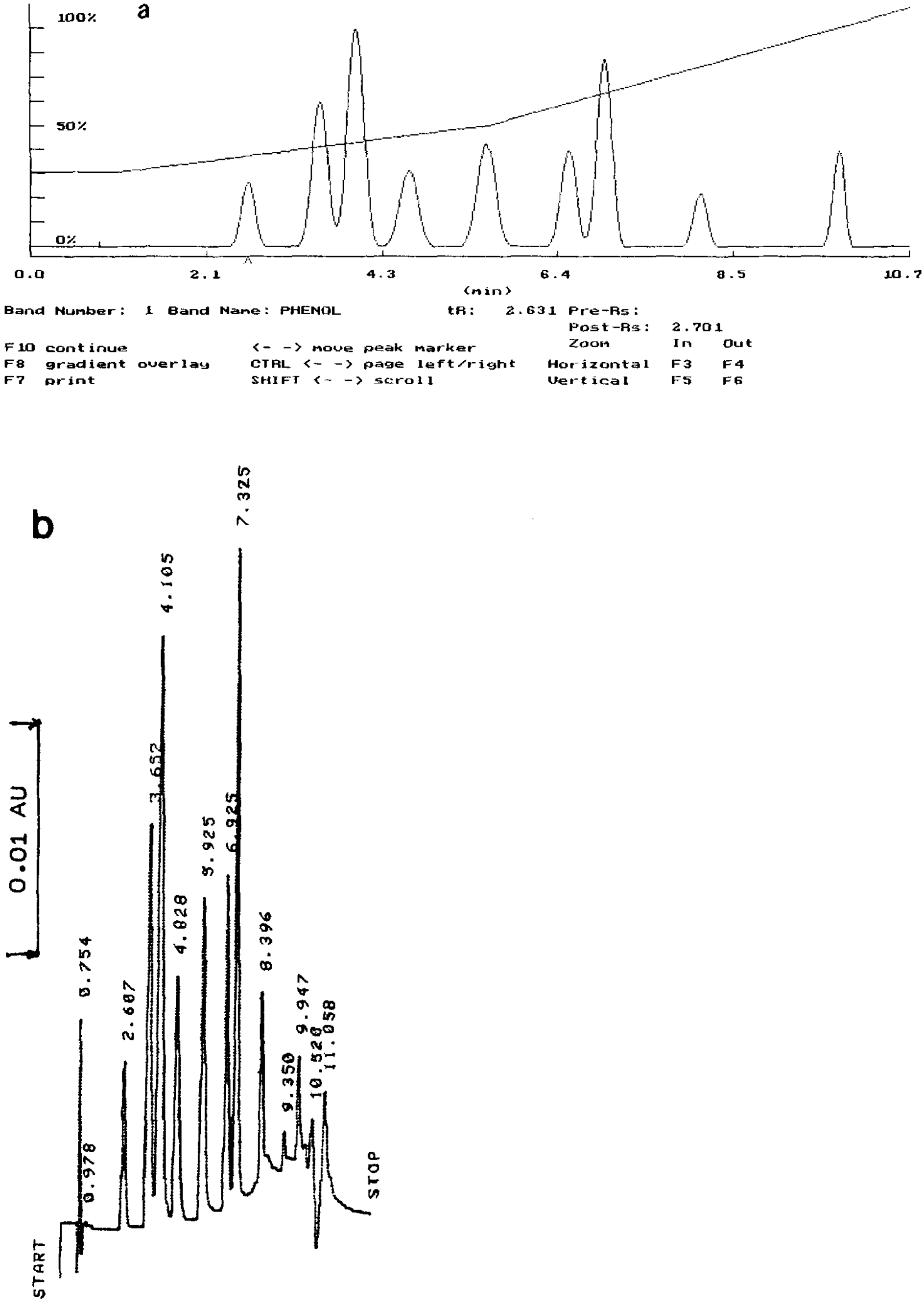


```
Band Number:  1 Band Name: PHENOL          tR:   2.631 Pre-Rs:
                                                         Post-Rs:  2.768
F10 continue         <- -> move peak marker          Zoom      In   Out
F8  gradient overlay CTRL <- -> page left/right   Horizontal   F3   F4
F7  print            SHIFT <- -> scroll           Vertical     F5   F6
```

Fig. 2 (a) Computer-simulated chromatogram (Drylab G). Sample as in Fig. 1a and b. The gradient is indicated by the dashed curve. (b) Experimental chromatograms. Sample as in Fig. 1a and b and conditions as in (a). (c) Blank gradient run with methanol–water at 280 nm. (d) Computer-simulated chromatogram (Drylab G). Sample as in Fig. 2a. The gradient is indicated by the dashed curve. The column efficiency decreased to $N = 1900$.



```
Band Number:  1  Band Name: PHENOL          tR:   2.631 Pre-Rs:
                                                        Post-Rs:   2.701
F10 continue            <- -> move peak marker          Zoom       In    Out
F8  gradient overlay    CTRL <- -> page left/right     Horizontal  F3    F4
F7  print               SHIFT <- -> scroll             Vertical    F5    F6
```

Fig. 3. (a) Computer-simulated chromatogram (Drylab G). Conditions as in Fig. 2a except flow-rate = 2 ml/min and the column efficiency decreased to N = 1008. (b) Experimental chromatogram. Conditions as in (a).

TABLE IV

RESULTS FOR OPTION 3: DATA FOR PRESENT SEPARATION FOR NINE BANDS IN A 31.0–100.0% MULTI-SEGMENTED GRADIENT RUN OVER 10.0 min IN TWO SEGMENTS

Gradient: 0 min, 31.0% B; 4.5 min, 50% B; 10 min, 100% B.

Band No.	$t_{R(calc.)}$	$t_{R(exp.)}$[a]	Band width	R_s[b]
1	2.68	2.607	0.151	5.38
2	3.55	3.652	0.172	2.48
3	3.98	4.105	0.180	3.45
4	4.63	4.828	0.194	4.84
5	5.57	5.925	0.195	5.39
6	6.54	6.925	0.166	2.60
7	6.97	7.325	0.162	7.45
8	8.13	8.396	0.150	11.96
9	9.81	9.947	0.131	

[a] Average error in t_R = ±0.22 min (= ±3.7%).
[b] Average in R_s (difference in t_R) = ±9%.

confirmed the accuracy of the predictions (Table IV) of retention times, the experimental R_s values being lower. When the R_s value of the critical pair was decreased to 1.50, even better agreement was obtained (Fig. 2d).

The conditions were further optimized by changing the column parameters. Retaining the same column (length, particle size), the flow-rate was changed to 2.0 ml/min. This resulted in some decrease in column efficiency (to N = 1008), which Drylab G took into account from its routine Knox relationship. The calcualted data are given in Table IV and the simulated and experimental chromatograms in Fig. 3. Here also the agreement is good; the time of analysis was reduced to 10 min.

CONCLUSIONS

The conditions for the gradient HPLC of phenol and eight derivatives were optimized using the Drylab G computer program. only a few experimental results were needed to choose a gradient programme that gave a satisfactory separation of all components in a short time. Good resolution of most pairs of of components indicated that the method is suitable also for the separation of mixtures containing varying relative amounts of the components. The chromatograms simulated by the computer were very similar to the experimental chromatograms.

ACKNOWLEDGEMENT

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