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REVIEW

COMPUTER-AIDED OPTIMIZATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN THE PHARMACEUTICAL INDUSTRY

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1. INTRODUCTION

The purpose of any chromatographic analysis is to separate a mixture into individual components for quantitative and qualitative determination. In order to obtain adequate separations of all compounds of interest in an acceptable analysis time, it is necessary to optimize the operating conditions.

In the last few years, many efforts have been made to develop systematic optimization strategies for high-performance liquid chromatography (HPLC). These strategies can be classified into two categories: one considering primarily mobile phase parameters such as solvent selectivity, solvent strength, pH and ion strength and the other using column parameters such as column length, column diameter, particle size of the packing material and eluent flow-rate for the fine tuning of the optimum conditions. Excellent overviews of method development in HPLC have been published[1–3].

Increased emphasis on HPLC method development and optimization has recently led to the use of computers in method development. The computer can be used to automate various phases of the HPLC process or to fit the retention data to various models in order to find the best conditions for a particular separation. Ideally, method development should be performed without restrictions regarding the number or the choice of parameters to be investigated. Owing to a mostly fragmental knowledge of the quantitative details of retention processes, empirical procedures are preferably

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used for this purpose. The globally optimum conditions can reliably be localized by the use of a sequential optimization strategy and an algorithm for non-linear interpolation between experimentally obtained retention data, even when multi-model response characteristics are encountered[4].

2. EXPERIMENTAL

2.1. Equipment and software

The HPLC system was a Kontron (Zurich, Switzerland) liquid chromatograph, consisting of three D-420 pumps, a D-460 autosampler equipped with a 100-ml Hamilton dosing syringe, two LMV-470 motorised low-pressure six-port valves and an M-800 mixing chamber. The pumps, six-port valves and autosampler were computer controlled via a multiport. The detector was Perkin-Elmer (Norwalk, CT, U.S.A.) LC-235 diode array with a flow-cell path length of 10 mm and a total volume of 4 ml, connected to a Perkin-Elmer GR-100 graphic printer-plotter. For pH measurements an Orion Research (Cambridge, MA, U.S.A.) SA 520 Metar was used.

Computer simulations were carried out using two types of software, DryLab I (LC Resources, Lafayette, CA, U.S.A.) and the program OPTIM[4], which was developed at the Technical University of Graz. For the three-dimensional representation of the optimization hypersurface the program Statgrafics (STSC, Rockville, MD, U.S.A.) was used.

2.2. Reagents and solvents

Acetonitrile, methanol and tetrahydrofuran (THF) were of HPLC grade (Rathburn, Walkerburn, U.K.). Water was deionized and filtered. Specific pH values were achieved using phosphate buffer (Merck, Darmstadt, F.R.G.). A cyano (5 μm) column (10 cm $\times$ 0.40 cm I.D.) (Knauer, Bad Homburg, F.R.G.) was used.

Synthetic mixtures of active compounds (Fig. 1) were prepared in-house.

2.3. Optimization approach

The aim of our optimization strategy is to find the best and the most rugged separation conditions (concerning solvent and column parameters) with the minimum number of experimental runs. To achieve this aim we combined two approaches of computer-aided optimization.

In previously conducted experiments with a factorial design approach, including the solvent selectivity triangle concept, 36 experimental runs were performed[5]. Such a large number of experimental runs could be performed overnight with our instrumental set-up (Fig. 2). Using Kontron software on the D450 data station, it was possible to program automatic switching of the two six-port valves and deliver different mobile phases to the system. This instrumental set-up allowed us to monitor mutual influences of parameters such as pH, ion strength and organic phase composition on the separation process. It is not easy, however, to find the best and the most rugged method when dealing with parameters such as pH and ion strength for ionic samples. Small variations in pH are known to produce enormous changes in retention[2], often with reversal of the elution order. The experimental data were used to find the global maximum for a given chromatographic response function (CRF)[6].

The retention behaviour of the individual species were processed by the OPTIM

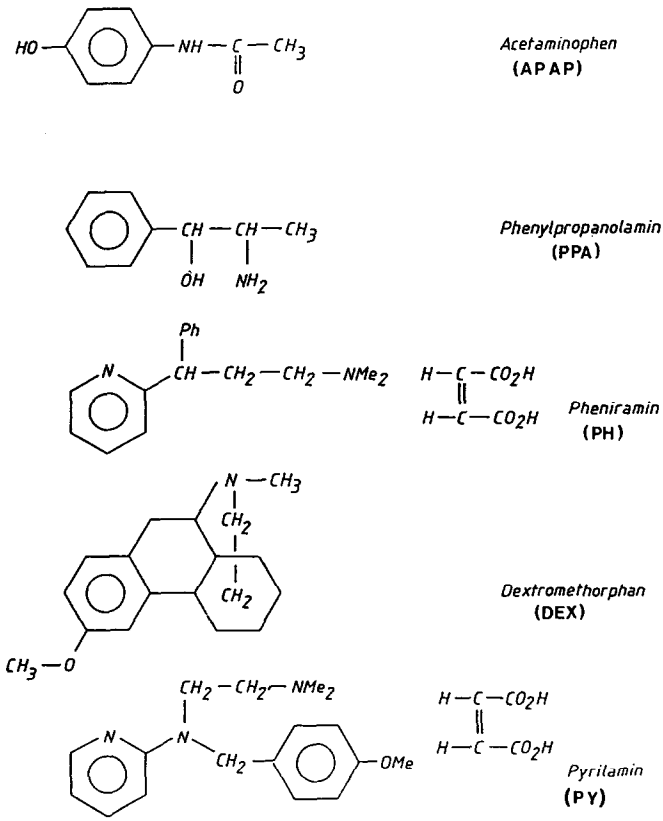

Fig. 1. Active components of Tussagesic tablets.

program in a such way that the mutual influences of the chromatographic variables on the CRF can be presented as a three-dimensional graph (hypersurface). The hypersurface is calculated using a moving least-square interpolation between experimentally obtained retention values. The optimum CRF conditions determined this way were used as the setting for the next two isocratic runs with different mobile phase compositions. The fine tuning of the mobile phase and column parameters was done with the data from those experiments. Proved chromatographic theory programmed in DryLab I[2] was used to visualize the influence of the elution strength in the mobile phase for isocratic conditions. Then the column parameter optimization was performed. In order to maximize the efficiency of the system, different column flow-rates, particle sizes and column lengths were simulated using column optimization section of the DryLab I program. Subsequently a chromatographic run was performed to validate the simulated data.

3. RESULTS AND DISCUSSION

In reversed-phase HPLC, the interaction between sample and solvent molecules is largely responsible for retention and selectivity phenomena. The nature of the

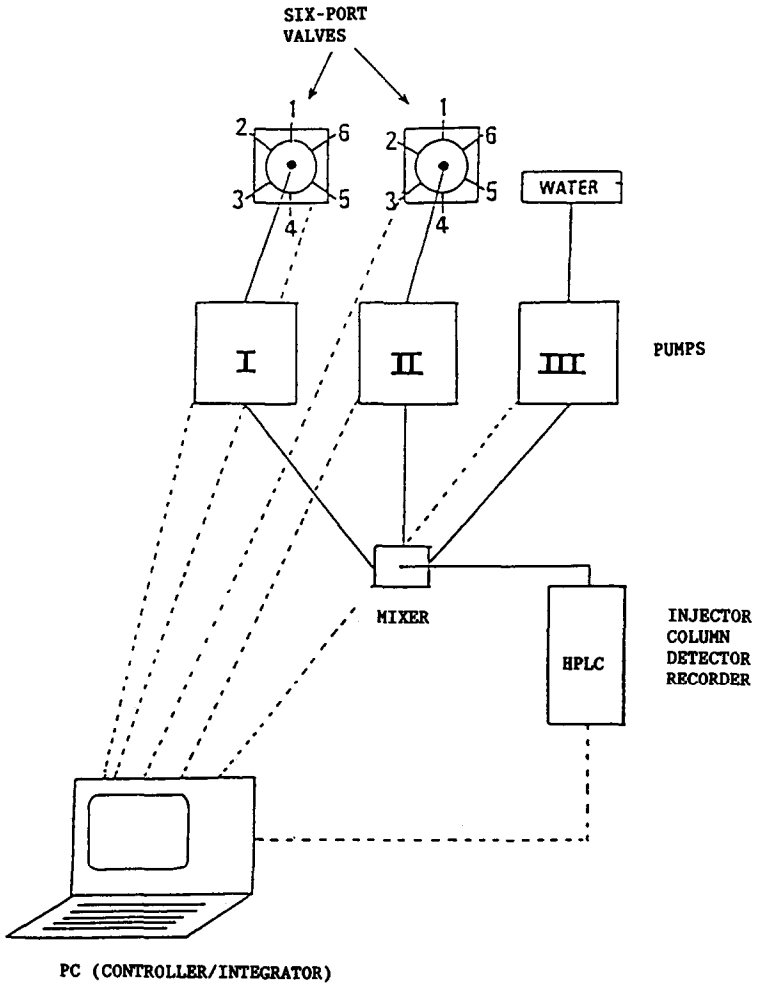

Fig. 2. Schematic diagram of the instrumental set-up. PC = Personal computer.

sample constituents and the separation system chosen for an adequate analytical characterization of the analytes determine the number and the nature of the variables available for the optimization process. The active compounds in the Tussagesic tablets (Fig. 1) are relatively polar and contain ionizable functional groups. Hence, the pH of the mobile phase is expected to play an important role in the separation process. Additionally, dipole–dipole and hydrogen-bonding interactions are considered to exhibit a substantial influence on the separation selectivity. In our study, solvents were classified according to their relative dipole moment, basicity and acidity, based on the concept of the solvent selectivity triangle[7].

The isocratic k' (capacity factor) should be in the range $1 < k' < 20$. Starting conditions of a binary mixture [acetonitrile–water (50:50) and buffer at pH 7] did not produce a complete separation; bands 3, 4 and 5 were unresolved. Changing the ratio of the solvent with a high elution strength (acetonitrile) to a weak eluting solvent

(water) did not improve the separation. This binary solvent composition did not yield a successful separation of components 3, 4 and 5. In an effort to change the separation selectivity, methanol–water mixtures were our next choice as the mobile phase. Adjusting for the same ionic strength as used previously with the acetonitrile–water system, the mixture of methanol–water (60:40) (buffer pH 7) was used. No significant improvement in separation was achieved. Finally, THF–water (38:62) (buffer pH 7) was used. This binary mixture led to an incomplete separation of bands 3, 4 and 5. Next, another isocratic run was performed with THF–water (60:40) and an attempt was made to optimize the binary mixture for an isocratic run using the Drylab I program. Owing to a strong dependence of the elution order of bands 3, 4 and 5 on the content of THF, computer simulation with this program did not reveal a binary mobile phase composition that would allow the complete separation of all components in the sample mixture.

As binary solvents did not yield a complete separation, a ternary mobile phase, consisting of a mixture of two organic solvents (acetonitrile and THF) and water was next applied. We used our in-house instrumental set-up, which allowed a large number of experiments to be performed overnight. As depicted in Fig. 2, pump I delivered organic phase with different acetonitrile:THF ratios and pump II was used to supply buffers with different pH values. Pump III delivered water. Six different organic compositions (70–95% acetonitrile in increments of 5%) were combined with six different buffers (varying from pH 5.5 to 8.0 in increments of 0.5). We examined the influence of the amount of THF in the ternary mobile phase and also the influence of pH on the band elution. These experimental results (representative chromatograms are depicted in Figs. 3 and 4) were used as input for the OPTIM program, using the CRF as criterion of the optimization. The results for CRF are represented graphically as a hypersurface in Fig. 5. A careful inspection of the response revealed that the highest CRF value is obtained at an acetonitrile:THF ratio of 9:1 and pH 7. Hence the most

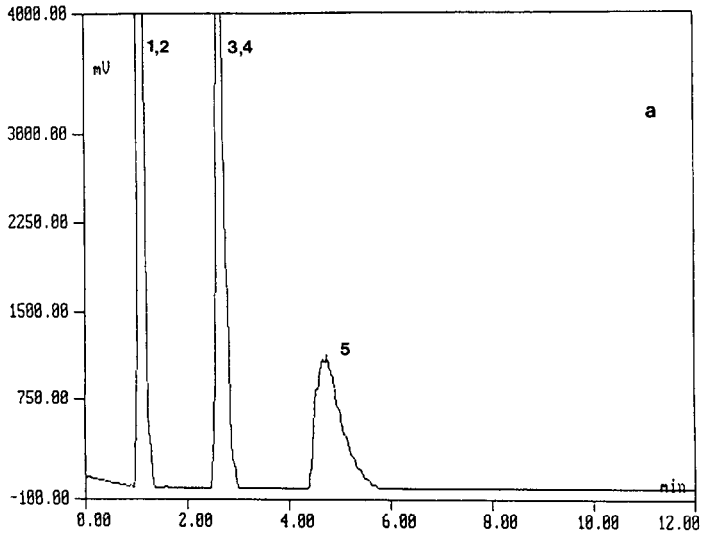

Fig. 3.

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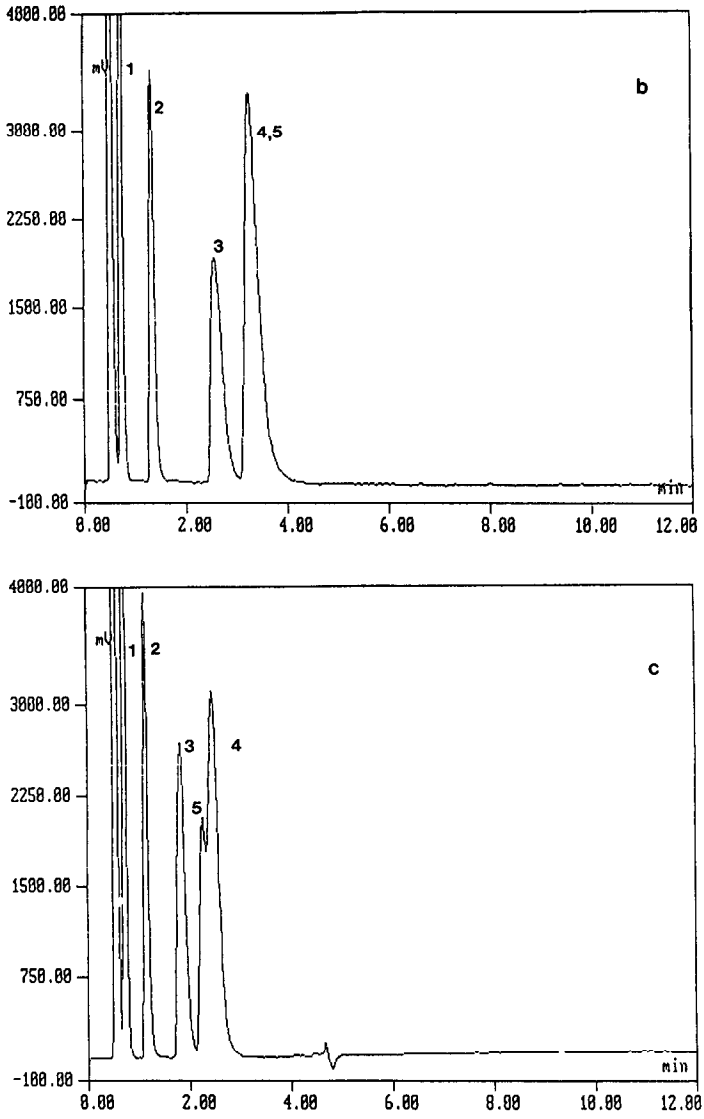

Fig. 3. Effect of mobile phase pH on band spacing. Cyano (5 μm) column (10 × 0.4 cm I.D.). Mobile phase: organic phase [acetonitrile–THF (9:1)]–water (50:50) at (a) pH 5.5, (b) pH = 6.5 and (c) pH 7.5; flow-rate, 1.5 ml/min. Peaks: 1 = APAP; 2 = PPA; 3 = PH; 4 = DEX; 5 = PY.

critical phase of the optimization procedure, the localization of the experimental optimum at conditions that are not sufficiently predictable by straightforward chromatographic theory, could be handled by the use of the fully empirical optimization scheme.

For fine tuning of the results, two isocratic runs, with 50% and 30% of organic solvent [acetonitrile–THF (9:1)] in water and at pH 7, were performed (Fig. 6). The

data were then used with DryLab I for the final optimization of the mobile phase for isocratic elution conditions. In Fig. 7 the limiting chromatographic resolution is plotted against percentage of organic modifier. Two percentages of the organic phase giving high resolution, namely 50 and 70%, were found. We chose 70% as the optimum composition for two reasons. First, there is a small plateau, meaning that a small change in the percentage of organic phase will not significantly affect the resolution and second, the analysis time is shorter. Finally, retention data obtained with this mobile phase composition were used for optimization of the column parameters, restricted to flow-rate optimization, using the DryLab I software. An optimum flow-rate of 1.4 ml/min with a mobile phase consisting of organic

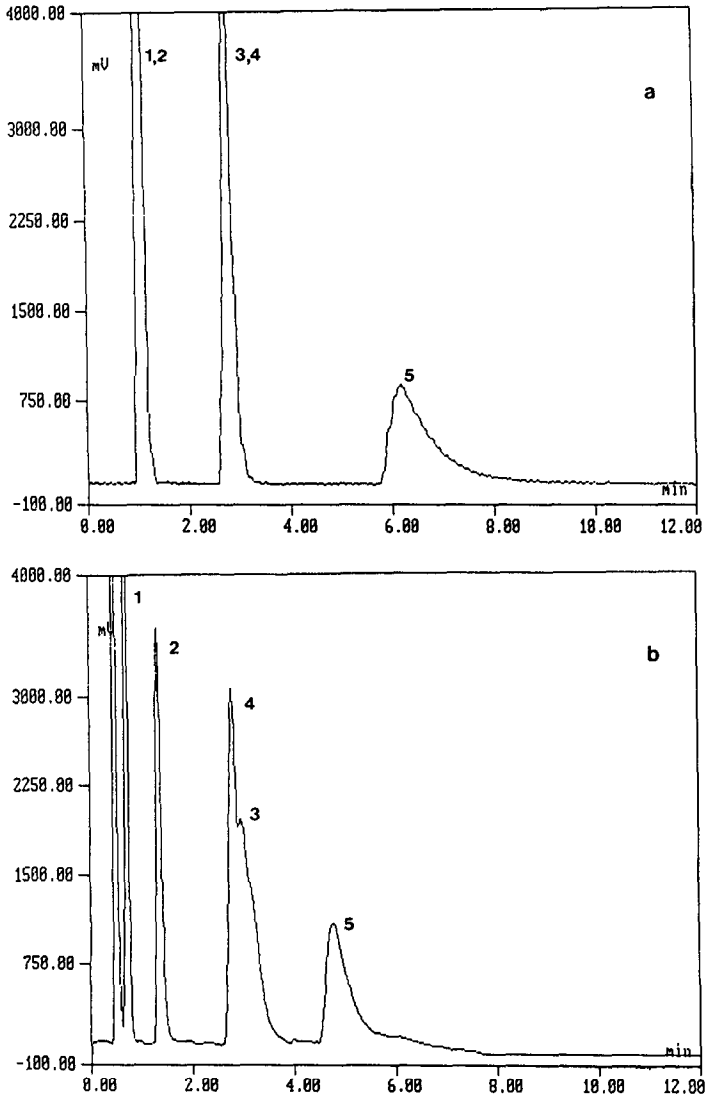

Fig. 4.

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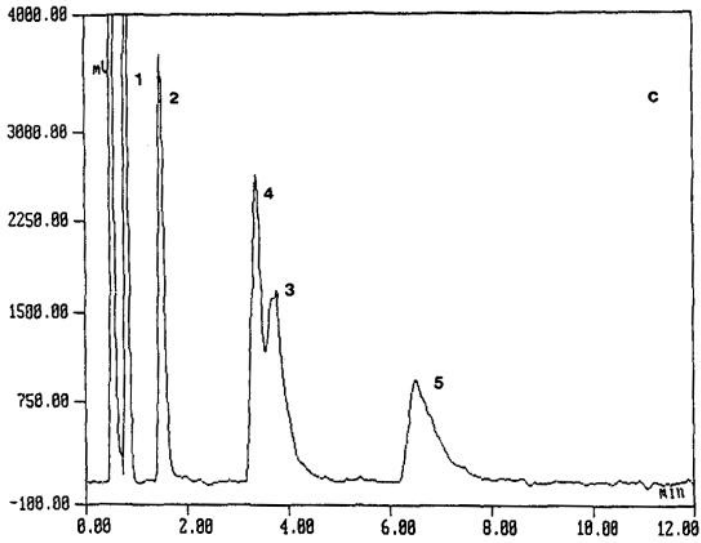

Fig. 4. Effect of the ternary mobile phase composition on retention. Mobile phase: organic phase–water (50:50) (pH 8). Ratio of acetonitrile to THF in organic phase: (a) 9.5:0.5; (b) 9:1; (c) 8.5:1.5. Flow-rate, 1.5 ml/min. Peaks as in Fig. 3.

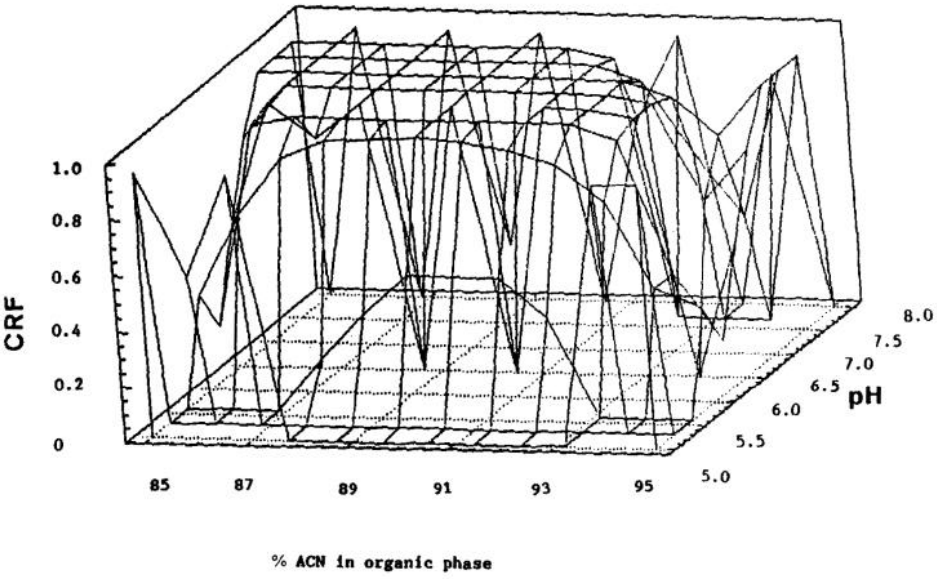

Fig. 5. Hypersurface obtained with a sequential approach using data from 36 experiments. ACN Acetonitrile.

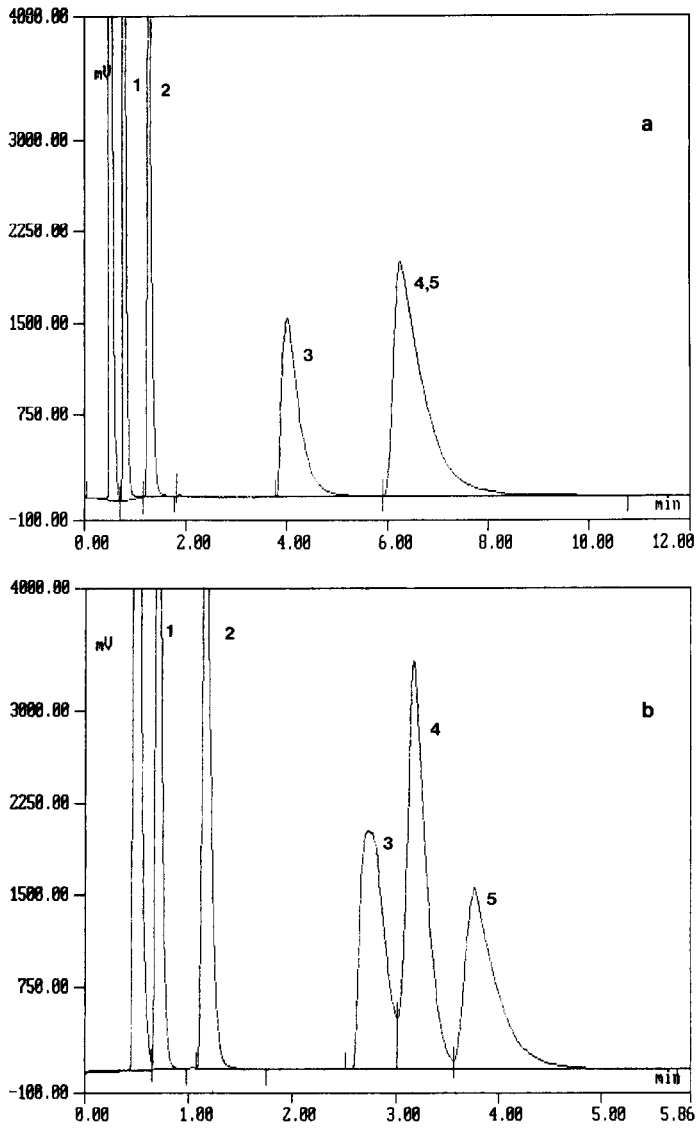

Fig. 6. Isocratic runs at pH 7 and acetonitrile: THF = 9:1; (a) organic phase–water (50:50); (b) organic phase–water (30:70). Peaks as in Fig. 3.

phase–water (70:30) (with buffer of pH) was used and confirmed by the run shown in Fig. 8. These optimized conditions are well suited for the reproducible determination of all constituents in Tussagesic tablets.

It is essential, however, for the use of both OPTIM and DryLab to attribute the chromatographic signals correctly to the analytes during the progress of the optimization. This identification was performed by separate injections of standards.

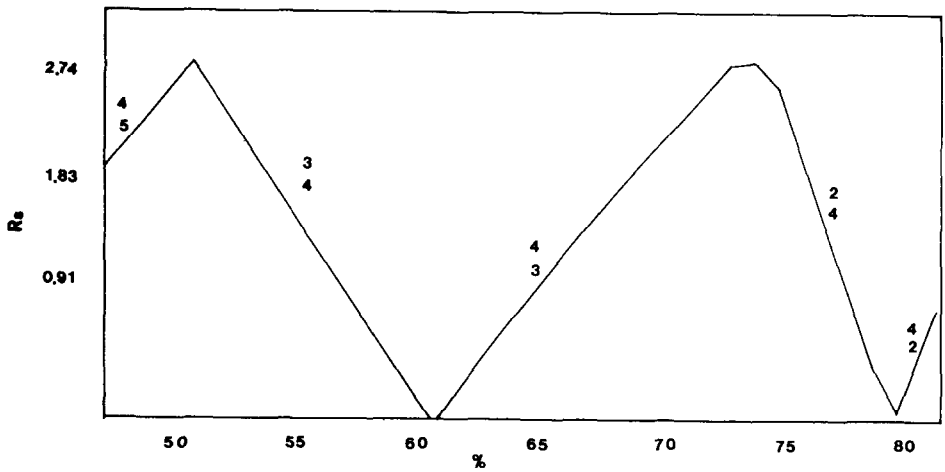

Fig. 7. Relative resolution mapping (resolution, R_s, *vs.* percentage of organic phase) for the five components of Tussagesic tablets.

The application of an automated peak recognition routine based on fuzzy algebra[8] is currently under investigation.

4. CONCLUSION

It was demonstrated that HPLC conditions can be optimized under the conditions required in the pharmaceutical industry. The nature of the experiments

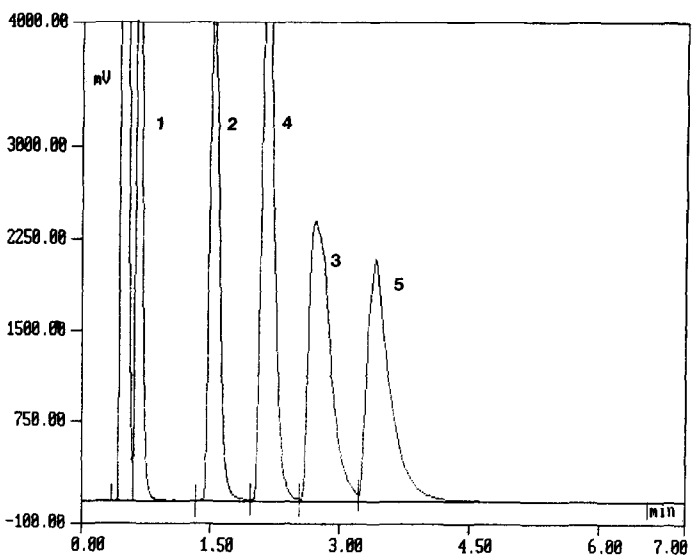

Fig. 8. Isocratic run with organic phase [acetonitrile–THF (9:1)]–water (70:30) at pH 7; flow-rate, 1.4 ml/min. Peaks as in Fig. 3.

demonstrates that empirical modelling of retention data is best suited to meet the requirements of the unpredictable elution behaviour of analytes. This reflects a typical situation in method development in the environment of the pharmaceutical chemistry. The graphical presentation of the critical parameters with an optimization hypersurface helps considerably in judging the ruggedness of a separation system. Moreover, inter-laboratory method transfer and documentation of method development should be facilitated by this procedure. A simultaneous consideration of chromatographic theory is very useful for the adjustment of column and solvent parameters.

5. SUMMARY

Optimization of high-performance liquid chromatography for application to a cough medication (Tussagesic) and its decomposition and byproducts was performed. Special emphasis was placed on the optimization of all parameters that relate to the chemical selectivity of the separation process itself and on the final proof of the ruggedness of the optimized system. All analytes can be reliably determined and the response surface can be represented graphically. This provides a means for improved transfer of methods between laboratories and for efficient system documentation.

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