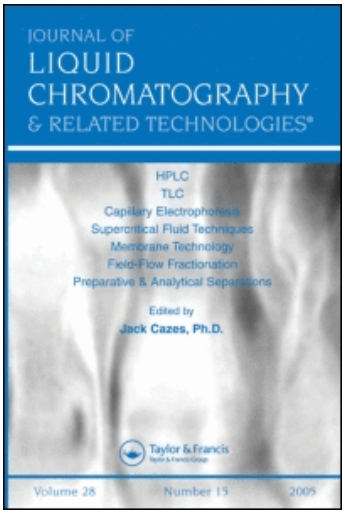

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HPLC Method Development for Duloxetine Hydrochloride Using a Combination of Computer-Based Solvent Strength Optimization and Solvent Selectivity Mixture Design

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HPLC METHOD DEVELOPMENT FOR DULOXETINE HYDROCHLORIDE USING A COMBINATION OF COMPUTER-BASED SOLVENT STRENGTH OPTIMIZATION AND SOLVENT SELECTIVITY MIXTURE DESIGN

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ABSTRACT

Computer simulation software for solvent strength optimization and statistical mixture design based on the solvent selectivity triangle were useful tools employed for the development of a reversed-phase HPLC method to separate duloxetine, a new anti-depressant compound, and structurally-related impurities. Solvent strength optimization was used to show that adequate separation for all impurities could not be obtained with a single organic modifier and to aid in choosing appropriate boundary conditions for a mixture design study. The mixture design was used to obtain resolution maps for organic modifier mixtures consisting of acetonitrile, methanol, and tetrahydrofuran. Overlapping resolution maps for the peak pairs of interest revealed the solvent composition that would provide the maximum resolution. Finally, solvent strength was optimized at the best solvent composition and information about method robustness obtained.

INTRODUCTION

Many computer-aided techniques for the development and optimization of high performance liquid chromatographic (HPLC) methods have been described.[1-8] Two of the more successful and widely-employed methods are solvent strength optimization using computer simulation,[9-14] and solvent selectivity optimization using a statistical mixture design.[15-26] With the solvent strength optimization technique, isocratic separations at various solvent strengths can be simulated after obtaining data from two gradient runs with different gradient slopes.

A disadvantage of this method is that only selectivity advantages derived from different solvent strengths are obtained. Different organic modifiers, or mixtures of modifiers, must each be treated as separate optimization experiments. No predictions are available for modifier combinations that have not been tested. This disadvantage, however, is the strength of solvent selectivity optimization using a mixture design approach.[15] With this technique, seven experiments from a statistical mixture design are performed using three different organic modifiers such as acetonitrile (ACN), methanol (MeOH), and tetrahydrofuran (THF).

Capacity factor and/or resolution data may then be mapped for any combination of organic modifiers and the optimum isocratic conditions chosen. The disadvantage of this technique is that no information about the separation at different solvent strengths other than those bounded by the experiment is obtained.[19] Also, a poor initial choice of boundary conditions for the mixture design can lead to suboptimal results.

In this paper, a combination approach to HPLC method development taking advantage of the complementary strengths of the solvent strength and mixture design selectivity techniques is described. The method development problem involved the separation of process-related impurities and degradation products in duloxetine hydrochloride, a serotonin/norepinephrine reuptake inhibitor currently undergoing clinical trials for the treatment of depression and urinary incontinence.

Structures of duloxetine and potential impurities are given in Figure 1. Compounds 4 and 5 are potential impurities from the synthetic process while 2, 3, and 6 are degradation products. Compounds 2 and 3 result from cleavage of the naphthyl ether and rearrangement to give the substituted naphthols. The initial cleavage products containing only the thiophene ring and aliphatic side chain were well-separated early in the chromatograms and were not included in the optimization.

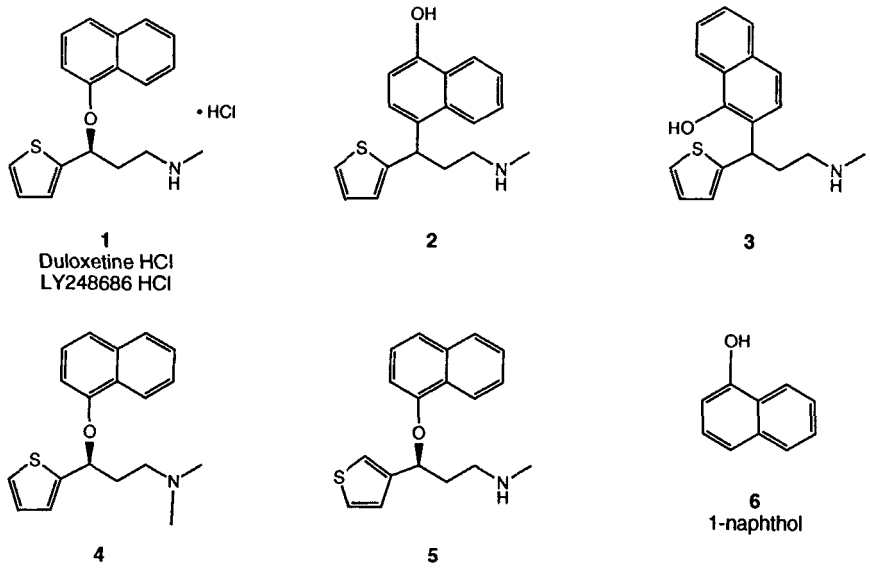

Figure 1. Duloxetine and potential impurities

- Perform two gradient runs with each organic modifier and simulate resolution vs. solvent strength
- For each modifier choose the optimum solvent strength from simulation results
- Perform 7-experiment mixture design study
- Generate resolution maps for peaks of interest
- Choose optimum solvent composition considering resolution and run time
- Perform two gradient runs using chosen modifier ratio
- Check ruggedness of separation and opportunities for optimization using solvent strength simulation

Figure 2. Combination HPLC method development approach employing solvent strength optimization and solvent selectivity mixture design.

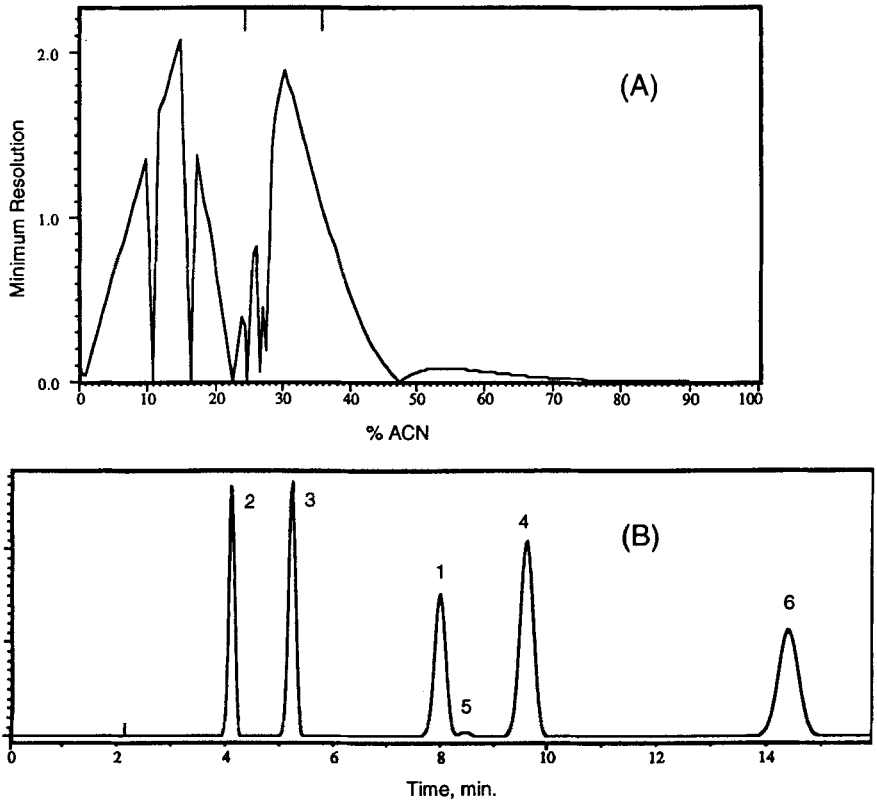

Figure 3. A) Resolution map for ACN modifier obtained using gradients from 20-50% ACN with gradient times of 20 and 40 minutes. Compounds 1 and 5 form the critical peak pair between 30 and 48% ACN. B) Chromatogram predicted for 35% ACN.

EXPERIMENTAL

Reagents

HPLC-grade acetonitrile, methanol, and tetrahydrofuran were obtained from EM Science (Gibbstown, NJ, USA). The mobile phase buffer was 50 mM potassium phosphate, pH 2.5, prepared using appropriate concentrations of potassium phosphate monobasic (EM Science), and orthophosphoric acid (85%, Fisher Scientific, Co., Fair Lawn, NJ, USA). The sample solvent was 30%

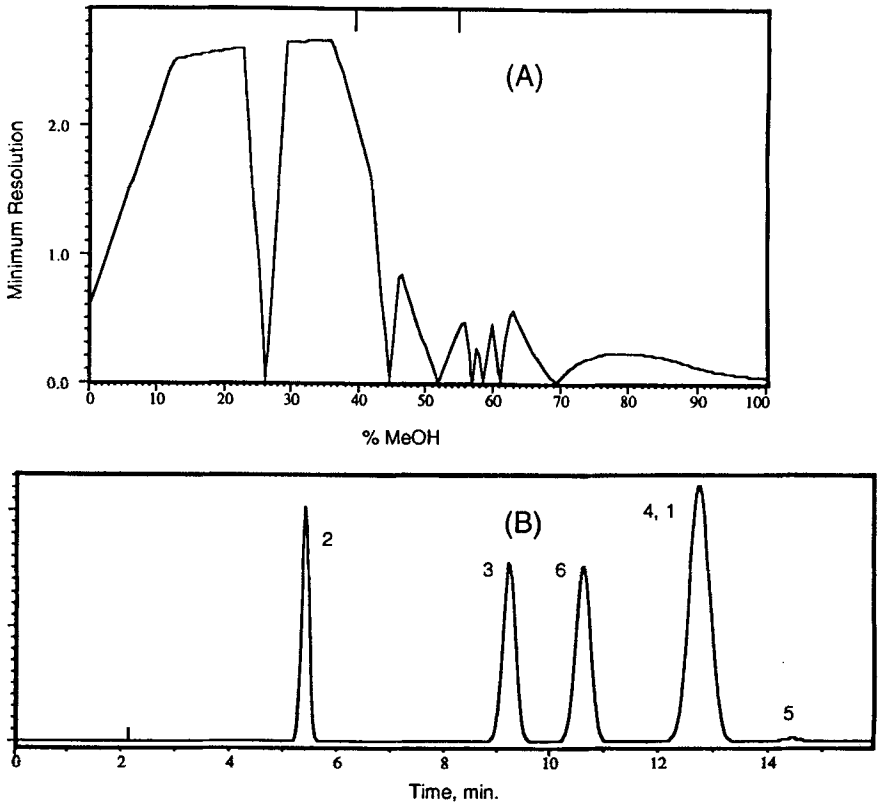

Figure 4. A) Resolution map for MeOH modifier obtained using gradients from 40-80% MeOH with gradient times of 20 and 40 minutes. Compounds 1 and 4 form the critical peak pair between 46 and 52% MeOH. B) Chromatogram predicted for 50% MeOH.

methanol in water. Water for mobile phases and sample solutions was purified with a Milli-Q system (Millipore, Milford, MA, USA). All mobile phase compositions are reported as volume/volume percentages of the aqueous buffer and organic modifiers.

Duloxetine hydrochloride and compounds 2-5 were from Lilly Research Laboratories. Compound 6, 1-naphthol, was obtained from Aldrich (Milwaukee, WI, USA) and recrystallized. Alternatively, compounds 2, 3, and 6 may be generated in solution by degrading duloxetine under acidic

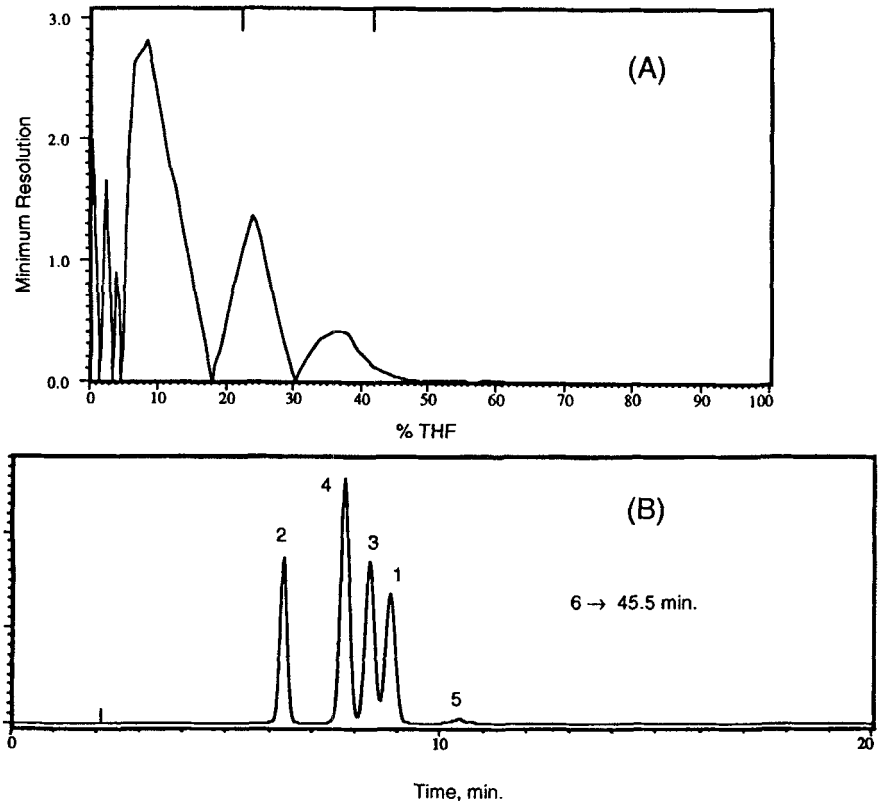

Figure 5. A) Resolution map for THF modifier obtained using gradients from 20-70% THF with gradient times of 20 and 40 minutes. Compounds 3 and 4 form the critical peak pair between 18 and 24% THF. Compounds 1 and 3 form the critical peak pair between 24 and 30% THF. B) Chromatogram predicted for 25% THF.

conditions. For example, a mixture of the degradation products was immediately formed upon addition of 0.1% v/v concentrated hydrochloric acid to a 0.1 mg/mL aqueous solution of duloxetine hydrochloride.

Apparatus and Conditions

The chromatographic system consisted of a Model 600 pump with column heater (Waters, Bedford, MA, USA), a Model 728 autoinjector (Alcott, Norcross, GA, USA) with a fixed-loop (10 µL) injection valve (Valco, Houston,

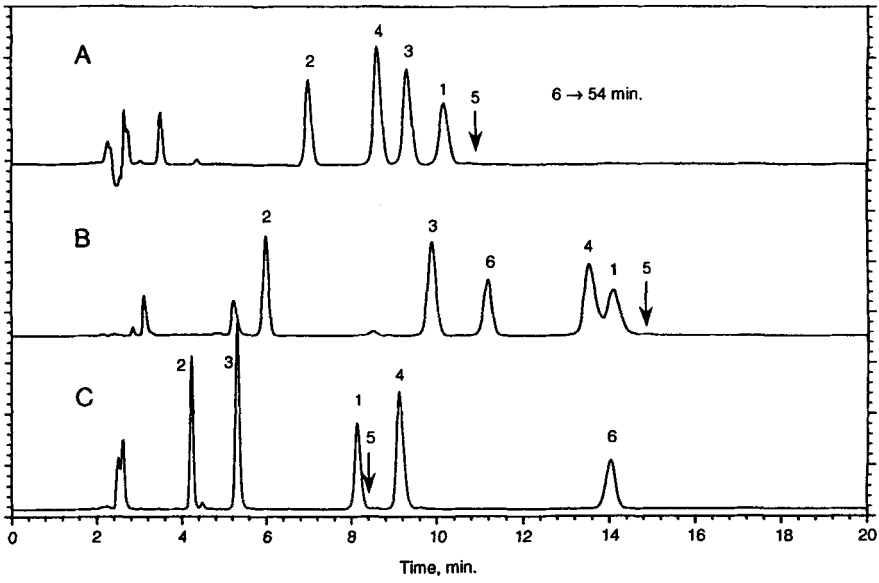

Figure 6. Chromatograms at vertex points of mixture design. A = 25% THF, B = 50% MeOH, C = 35% ACN. Retention of compound 5 indicated by arrows.

TX, USA), and a Model 787 variable wavelength UV detector set at 230 nm (Applied Biosystems, Ramsey, NJ, USA). Chromatograms were recorded using an in-house data acquisition system. A 250 mm x 4.6 mm ID, 5 μm particle size Zorbax RX-C_8 column (Mac-Mod Analytical, Chadds Ford, PA, USA) maintained at 35°C was used. The flow rate was 1.0 mL/min.

Software

DryLab G® software (version 1.53, LC Resources, Lafayette, CA, USA) was used for solvent strength optimization by calculating resolution versus solvent strength with data from two gradient runs for a given organic solvent. The statistical mixture design data were analyzed and resolution maps plotted with the JMP statistical software package (version 2.05 for the Macintosh, SAS Institute Inc., Cary, NC, USA). The overlapping resolution map was generated with a program written in QuickBASIC and plotted using Excel (Microsoft, Redmond, WA, USA).

RESULTS AND DISCUSSION

The combination development approach utilized in this study is outlined in Figure 2. Two gradient chromatograms for solvent strength studies are performed with each organic modifier: ACN, MeOH, and THF. Solvent strength optimization may show that one modifier will provide adequate results and no further development is needed. Results from these studies can also show whether selectivity changes using different modifiers warrant the use of a mixture design study and, if so, can aid in the choice of the individual modifier solvent strengths. If the retention order of peaks and their resolution values are relatively consistent for each modifier, solvent selectivity optimization may not be fruitful. If that is the case, pH optimization, other stationary phases, or modifiers such as ion-pairing reagents might be explored.

A low pH, where both the analytes and residual silanols on the stationary phase are protonated, was chosen for this study. Higher pH values might provide different selectivity but the separation may not be as rugged. Solvent boundary conditions can be chosen from the initial computer simulation results, and the mixture design is then conducted. Key peak resolutions are mapped to determine the optimum mobile phase composition for resolution. Optimization of analysis time may also influence the final choice of organic modifier conditions. Finally, the conditions chosen are then investigated for additional optimization and ruggedness using solvent strength modeling.

Retention data for duloxetine and impurities were obtained for two gradient runs using each organic modifier. Minimum resolution maps predicted by computer simulation are shown in Figures 3-5. Predicted isocratic chromatograms for each solvent at roughly equivalent solvent strengths are also shown. From these results, it was clear that relative peak retention varied greatly depending on the modifier used.

For example, the retention order of 4 and 1 was reversed between ACN and THF, while the peaks were coeluted with methanol at a comparable solvent strength. Also, 6 eluted before 1 with MeOH but after 1 with ACN, and it was very strongly retained (t_r = 45.5 minutes) with THF. The resolution between 1 and 5 with ACN was not sufficient to allow detection of small quantities of 5 (down to 0.1%) in the presence of 1 as the main component. Resolution of all peaks of interest was adequate with THF but the retention time of 6 was excessive.

The lack of acceptable results with a single modifier plus the significant differences in selectivity among the modifiers indicated that a solvent selectivity optimization should be performed. The following percentages of

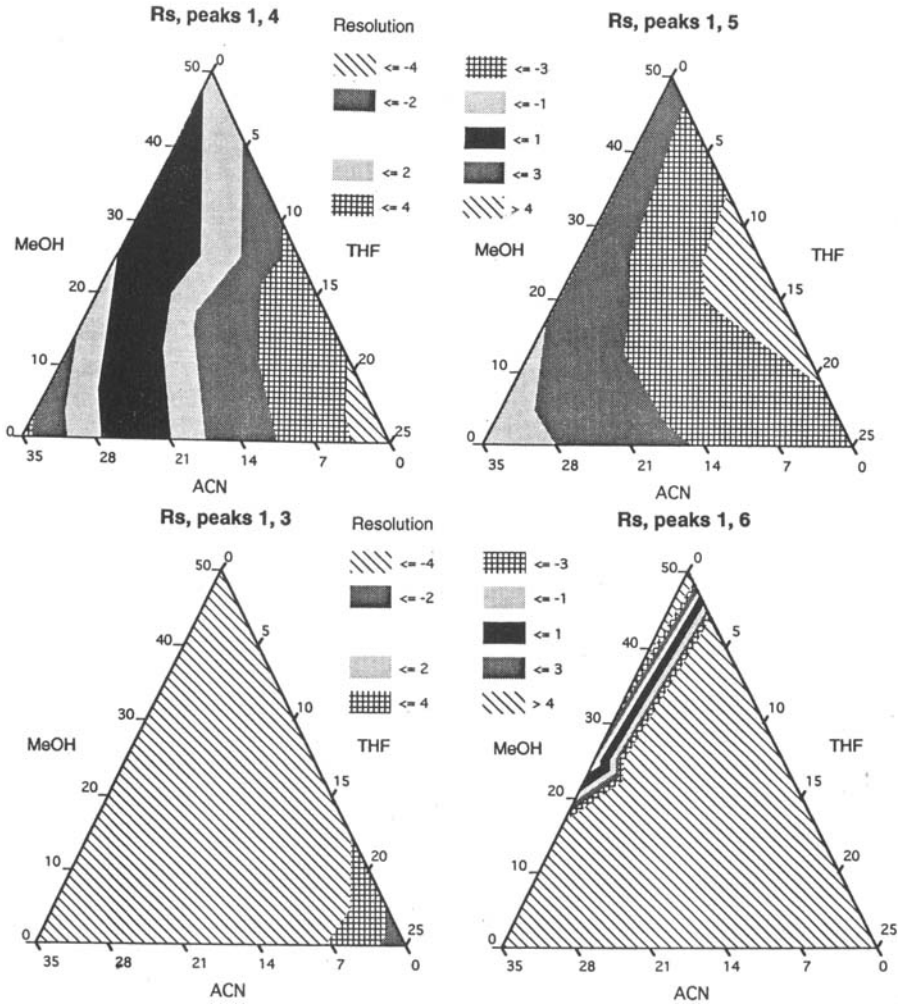

Figure 7. Resolution maps from solvent selectivity mixture design study.

each modifier were chosen for the mixture design study: ACN-35%, MeOH-50%, THF-25%. Although the percentages of ACN and MeOH were not those predicted to give maximum resolution, they allowed run times of less than 20 minutes without greatly compromising the resolution that was obtainable. It was not possible to maintain a reasonable resolution using only THF while keeping the run time under 20 minutes because of the long retention of 6.

Table 1

Solvent Selectivity Mixture Design Results

% Modifier			Resolution from Duloxetine[1]			
MeOH	**ACN**	**THF**	**5**	**4**	**3**	**6**
50	0	0	2.7	-1.2	9.6	-6.6
0	35	0	1.4	3.3	11.9	17.0
0	0	25	3.6	-4.5	2.5	33.4
25	17.5	0	2.3	1.0	12.5	-2.3
25	0	12.5	4.8	-3.4	5.1	36.8
0	17.5	12.5	2.9	-2.0	6.0	29.4
16.7	11.7	8.3	3.5	-2.4	7.6	17.6

[1]Resolution values are indicated as negative if impurity k′ is less than k′ of duloxetine.

The mixture design and resolution data from it are shown in Table 1. Resolution of impurities 3, 4, 5, and 6 from duloxetine were viewed as the key responses. Figure 6 shows chromatograms using single modifiers which correspond to the vertex points of the solvent selectivity triangle. The results agreed well with those predicted by simulation (Figures 3-5). Resolution maps for the four individual impurities from duloxetine, 1, are shown in Figure 7. Compound 3 was well-resolved under all conditions and 6 had only a narrow band of conditions producing poor resolution. Compound 5 had the lowest resolution from 1 in the region of high ACN modifier content, while compound 4 was not resolved over a significant portion of the selectivity map.

The best resolution conditions appeared to be toward the THF/MeOH axis and away from ACN. This was confirmed by an overlapping resolution map showing the minimum resolution for all four peak pairs over the range of solvent composition (Figure 8).

The following mobile phase composition was predicted to give a minimum resolution of 4.0: 2.5% ACN, 11% MeOH, 17.5% THF, and 69% buffer. While providing optimum resolution, the relatively high percentage of THF led to excessive retention of 6. Since MeOH provided decreased retention

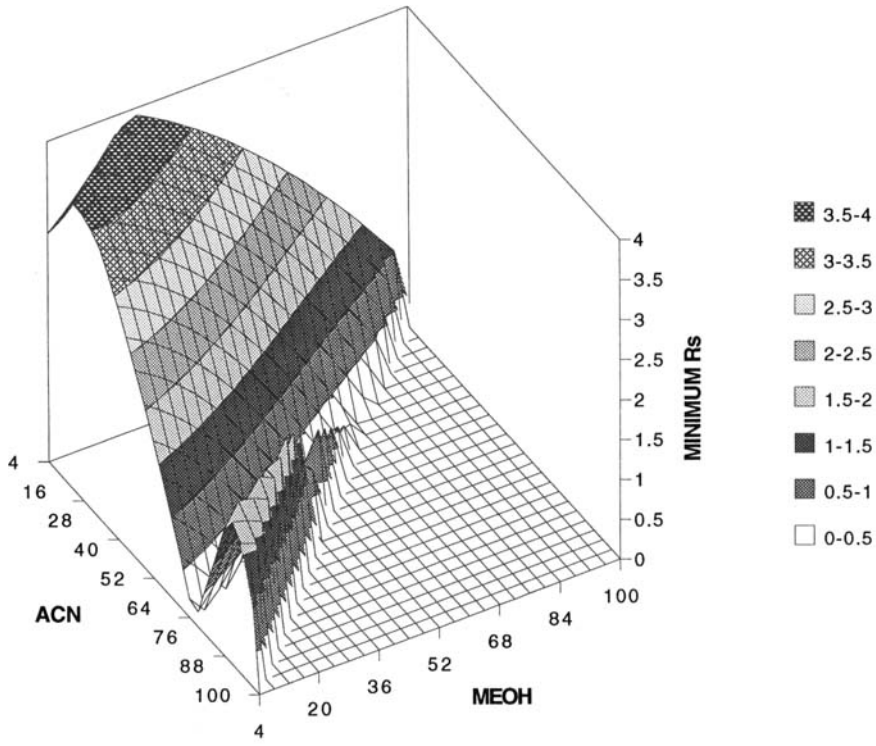

Figure 8. Overlapping resolution map from solvent selectivity mixture design study. Absolute MeOH and ACN percentages can be found by multiplying by 0.50 and 0.35, respectively. THF percentage can be found by subtracting ACN and MeOH (on graph) from 100 and multiplying by 0.25.

of 6 relative to other components, resolution predictions were obtained at increased MeOH concentrations. Also, ACN was eliminated to simplify the mobile phase. A composition of 25% MeOH, 12.5% THF was predicted to give a minimum resolution of 3.4 versus the optimum value of 4.0.

The run time was reduced even further by modifying the composition to 35% MeOH, 10% THF. This relative modifier ratio (35:10) was then used for final solvent strength optimization. Alternatively, a chromatographic response function such as that employed by Glajch et al. could have been used to simultaneously evaluate both run time and resolution during the solvent selectivity mixture design.[15]

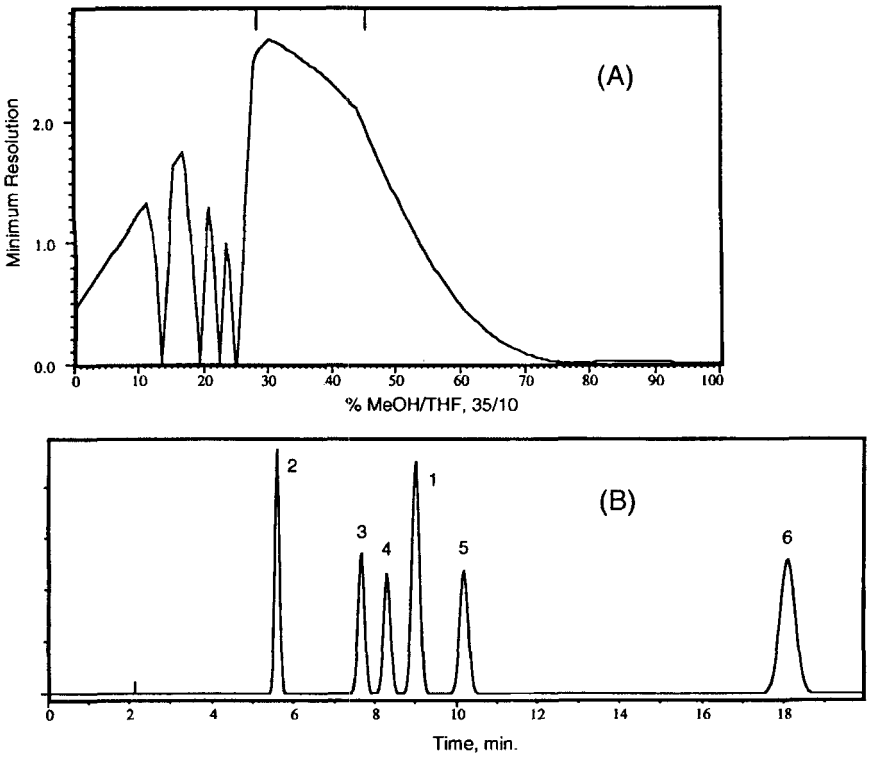

Figure 9. A) Solvent strength resolution map for MeOH/THF modifier obtained using gradients from 20-70% MeOH/THF, 35/10 with gradient times of 30 and 60 minutes. Compounds 1 and 4 form the critical peak pair between 30 and 44% MeOH/THF. Compounds 3 and 4 form the critical peak pair between 44 and 77% MeOH/THF. B) Chromatogram predicted for 45% MeOH/THF, 35/10.

Two gradient runs were performed using a mixture of MeOH and THF, 35/10, as the organic modifier. Figure 9 shows the minimum resolution map for this study. A simulated isocratic chromatogram at 45% of the MeOH/THF mixture (which corresponds to an overall mobile phase composition of 35% MeOH, 10% THF, and 55% buffer) shows greater than baseline resolution for all peaks from duloxetine with a run time of about 19 minutes.

Also, the resolution map is not steeply sloping over the solvent range of interest, indicating that the separation should be fairly rugged toward small

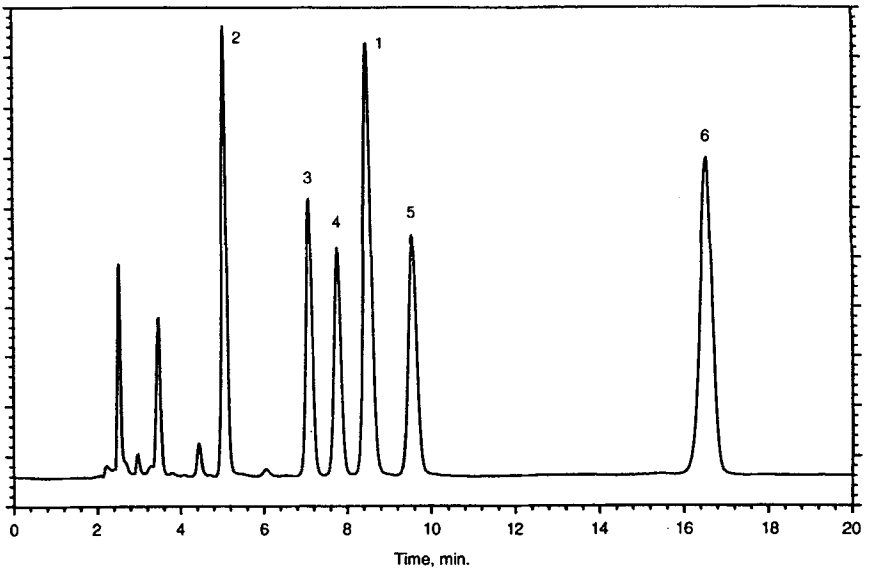

Figure 10. Experimental chromatogram at 44% MeOH/THF, 35/10.

changes in mobile phase composition. Slightly greater resolution could be obtained, if needed, by decreasing the MeOH/THF concentration, although the run time would lengthen. A chromatogram obtained using 44% of the MeOH/THF mixture is shown in Figure 10. Experimental retention times were about 10% less than those from the simulation which is within the agreement expected considering the accuracy of the simulation and mobile phase mixing.

CONCLUSIONS

The combination approach to mobile phase optimization provided rugged conditions which gave an acceptable separation of duloxetine from related impurities in under 20 minutes. In addition to indicating the mobile phase composition for optimal separation, the resolution maps from solvent strength simulations and the mixture design technique provide information about the separation ruggedness.

This information can also be used to adjust conditions appropriately to compensate for column or instrumental differences that may be encountered in the future.

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