Journal of Chromatography, 628 (1993) 191–198
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 636

Use of computer simulations in the development of gradient and isocratic high-performance liquid chromatography methods for analysis of drug compounds and synthetic intermediates

Loren Wrisley

Analytical Research and Services, Wyeth-Ayerst Laboratories, 64 Maple Street, Rouses Point, NY 12919 (USA)

(First received September 2nd, 1992; revised manuscript received September 22nd, 1992)

ABSTRACT

DryLab G/plus and DryLab I/plus (LC Resources) are shown to be effective aids in the development and optimization of gradient and isocratic HPLC conditions for the assay of drug substances and related compounds. Data obtained after two experimental runs in the laboratory are entered into the appropriate program where HPLC conditions can be altered (*e.g.* flow-rate, column dimensions, mobile phase composition, gradient steepness and shape, etc.) to arrive at optimum separation conditions with less analyst time required. The computer simulations from DryLab G/plus are shown to be suitably accurate under "real life" conditions in the development of gradient purity methods for two drug substances (Zalospirone and WY-47 384) and two synthetic intermediates (cyclooctatetraene and 2-methylcarboxybenzaldehyde). Moreover, DryLab I/plus was shown to be accurate in predicting isocratic retention for the separation of impurities in cyclooctatetraene, both in scaling down to small columns for speed and scaling up to a semi-preparative separation for isolation of impurities.

INTRODUCTION

Typically, the development of rugged HPLC separations involves a significant investment of time and effort because the variety of stationary phases and mobile phase combinations provides for a broad array of possible separation conditions from which to choose. To assist chromatographers in this endeavor, a variety of HPLC method development schemes have been described [1–4]. The potential utility of computer simulation software packages as tools to help guide chromatographers to appropriate separation conditions is significant.

DryLab computer simulation programs (LC Resources, Lafayette, CA, USA) were designed to help chromatographers optimize separation conditions using fewer actual experimental runs. These software packages for isocratic and gradient HPLC methods development have been well documented by Snyder and co-workers [5,6]. In brief, data from two initial separations (isocratic or gradient) are entered into the appropriate program, then simulated experiments can be carried out at the computer to determine the effects on the separation of changing conditions such as flow-rate, percent organic modifier, gradient time and shape, column dimensions, etc. While taking advantage of this computer simulation software for HPLC method development, a chromatographer should be able to save considerable time and develop better HPLC separations.

EXPERIMENTAL

Instrumentation

The HPLC system used for these studies was

Correspondence to: L. Wrisley, Wyeth-Ayerst Laboratories, 64 Maple Street, Rouses Point, NY 12919, USA.

0021-9673/93/$06.00

composed of the following: a dual pump gradient (Model 590 and Model 6000A, Waters Assoc., Milford, MA, USA), a programmable variable-wavelength UV detector/gradient controller (Spectroflow 783G, Applied Biosystems, Ramsey, NJ, USA), an autoinjector (Model 710B WISP, Waters), and integration system (Model HP-3356, Hewlett-Packard, Avondale, PA, USA). DryLab I/plus and DryLab G/plus software was supplied by LC Resources, and was operated on an IBM PC-XT computer. The total dwell volume of the gradient system was about 3 ml.

Reagents and materials

All drug substances, intermediates and known impurities were obtained in-house (Wyeth-Ayerst Research). HPLC-grade methanol and acetonitrile (J. T. Baker, USA) were used as is. Distilled water was treated with a Milli-Q purification system (Millipore, USA) before use. Methanesulfonic acid (Aldrich, USA) was vacuum distilled before use. μBondapak C_{18} (Phenomenex, USA), Spherisorb C_8 and ODS-2 (Phase Separations, USA), Ultrasphere ODS (Beckman, USA), Supelco LC-18DB (Supelco, USA) and Pecosphere C_{18} (Perkin-Elmer, USA) columns were used.

Procedure

To demonstrate the practicality of computer simulations in the optimization of reversed-phase HPLC methods, DryLab G/plus and I/plus were used to aid in the development of four HPLC methods designed to assess the purity of drug compounds or synthetic intermediates. Overall, the separation goals were defined with an emphasis on the rugged separation of the maximum number of peaks. Of secondary importance was the speed of the separation.

Appropriate HPLC solvents were prepared to give good peak shapes and efficiencies for the compounds of interest. These mobile phase choices were based either on previous HPLC experience with the compounds of interest (examples 1 and 4) or on a "best guess" basis given the expected chromatographic behavior of the test compounds (examples 2 and 3). Two linear gradients were then run for each test compound over the same gradient range and with the same flow-rate. The gradient time of the first run was set at 15–20 min, while the gradient time for the second run was 3–4 times longer. Peaks were tracked during the optimization experiments based on their relative areas. Retention time data for observed peaks from the pairs of gradient test runs were entered into the DryLab G/plus program along with other data concerning the separation (flow-rate, temperature, organic solvent, column dimensions, etc.). Separation conditions including gradient shape, range, flow-rate, column dimensions, etc., could all be manipulated within the program, essentially to run separation experiments for projecting optimum conditions.

After the computer simulations were completed, the optimum predicted conditions were experimentally validated. The theoretical predictions from the computer simulations were then compared to the actual HPLC chromatograms. When possible, peaks were identified following optimization using authentic standards.

RESULTS

Gradient optimization

The first test involved a purity assay for the experimental drug substance zalospirone which, in its crude form, could have a number of impurities including synthetic intermediates and by-products. The initial linear gradient trials are shown in Fig. 1A and B. Changes in peak resolutions and even elution order between the two trials are evident. Using the information from these trials, 14 DryLab experiments were carried out before the optimum separation conditions depicted in Fig. 1C were arrived at with a total time of about 40 min at the computer. This is significantly less than the approximately 9 h of instrument time that would have been needed to perform the actual experiments.

Table I shows a comparison between the DryLab G/plus computer predicted retention times for the computer-optimized gradient separation and the experimentally observed retention times. Despite some minor differences, the final separation shown in Fig. 1C appears to be quite suitable with good resolution between all peaks.

A second use of DryLab G/plus involved the separation of the experimental drug substance WY-47 384 from possible synthetic impurities. Using basically the same mobile phases and column as for zalospirone, initial gradient trials were run as

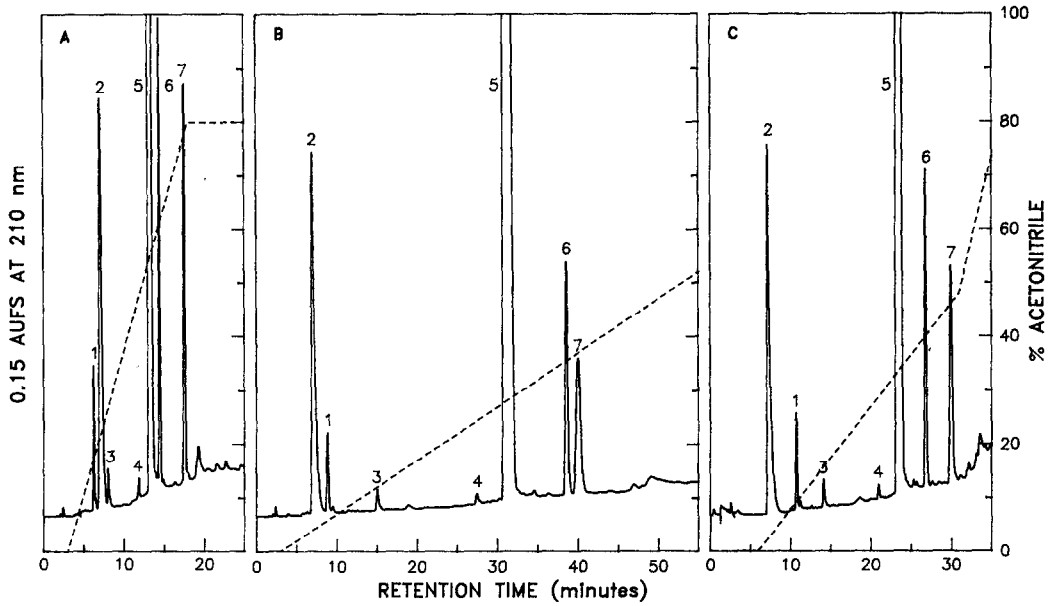

Fig. 1. (A, B) Results of initial gradient experiments run to acquire data for DryLab G/plus on crude zalospirone (peak 5). Mobile phase: 6 ml methanesulfonic acid/l water at pH 3.0 with KOH–acetonitrile. Column: 30 × 0.39 cm I.D. μBondapak C_{18}, 10 μm. Flow: 1.5 ml/min. Gradient: 0 to 80% acetonitrile in 15 min (A) and in 80 min (B). (C) Optimized gradient separation based upon DryLab G/plus predictions. Gradient: 0% acetonitrile for 3 min, then to 48% acetonitrile by 28 min, and to 80% acetonitrile by 33 min. Sample: 20 μl of crude zalospirone in methanol–dichloromethane (9:1). Peaks: 1 = synthetic precursor; 2 = dichloromethane; 3, 4, 7 = impurities; 5 = zalospirone; 6 = dimeric impurity.

shown in Fig. 2A and B. After 10 computer simulations were performed, an optimum gradient was arrived at as shown experimentally in Fig. 2C. The resolution between peak 2 and peak 3 (WY-47 384) is now very good with this optimized, segmented gradient. Table II compares the DryLab G/plus predicted retention times with found results.

Similarly, DryLab G/plus was utilized to develop a purity assay for 2-methylcarboxybenzaldehyde (2MCBA). Using acetonitrile–water, the initial gra-

TABLE I

COMPARISON OF ACTUAL RETENTION TIMES WITH DryLab G/plus PREDICTIONS FOR AN OPTIMIZED GRADIENT SEPARATION OF ZALOSPIRONE FROM IMPURITIES

Conditions: see Fig. 1.

Peak	Retention time (min)	
	DryLab	Actual
1	7.1	7.1
2	9.6	10.6
3	14.3	13.8
4	22.7	20.7
5	25.2	23.3
6	29.3	26.7
7	32.3	29.8

TABLE II

COMPARISON OF ACTUAL RETENTION TIMES WITH DryLab G/plus PREDICTIONS FOR AN OPTIMIZED GRADIENT SEPARATION OF WY-47 384 FROM IMPURITIES

Conditions: see Fig. 2.

Peak	Retention time (min)	
	DryLab	Actual
1	11.6	10.6
2	20.6	19.7
3	22.6	23.5
4	29.8	29.7
5	37.3	37.4

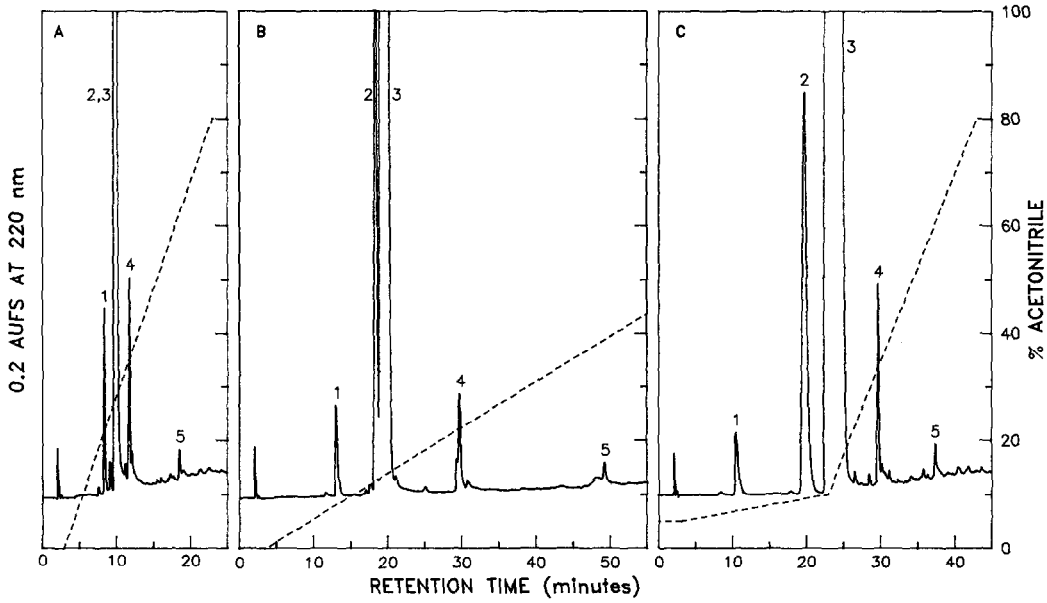

Fig. 2. (A, B) Results of initial gradient experiments run to acquire data for DryLab G/plus on crude WY-47 384 (peak 3). Mobile phase: 6 ml methanesulfonic acid/l water at pH 3.0 with KOH–acetonitrile. Column: 30 × 0.39 cm I.D. μBondapak C_{18}, 10 μm. Flow: 1.5 ml/min. Gradient: 0 to 80% acetonitrile in 20 min (A) and in 80 min (B). (C) Optimized gradient separation based upon DryLab G/plus predictions. Gradient: 5 to 10% acetonitrile in 20 min, then to 80% acetonitrile by 40 min. Sample: 20 μl of crude WY-47 384 in acetonitrile. Peaks: 1, 2 = synthetic precursors; 3 = WY-47 384; 4, 5 = synthetic by-products.

dient trials were performed as shown in Fig. 3A and B. Seven computer simulations gave the separation conditions used to generate the chromatogram shown in Fig. 3C which shows excellent resolution between impurity peaks 2 and 3 with the separation carried out in about 25 min. Comparisons between predicted and found retention times for this gradient separation are shown in Table III.

Finally, a method was developed to detect dimeric impurities in cyclooctatetraene. The initial methanol/water gradient trials (Fig. 4A and B) led to an optimized gradient shape shown in Fig. 4C after 8 computer simulations. Retention time comparisons are presented in Table IV.

TABLE III

COMPARISON OF ACTUAL RETENTION TIMES WITH DryLab G/plus PREDICTIONS FOR AN OPTIMIZED GRADIENT SEPARATION OF 2MCBA FROM IMPURITIES

Conditions: see Fig. 3C.

Peak	Retention time (min)	
	DryLab	Actual
1	8.6	10.9
2	11.7	13.2
3	12.6	14.2
4	15.1	15.8
5	17.2	17.7
6	18.6	18.9
7	23.0	23.2

TABLE IV

COMPARISON OF EXPERIMENTAL RETENTION TIMES WITH DryLab G/plus PREDICTIONS FOR THE OPTIMIZED GRADIENT SEPARATION OF COT FROM IMPURITIES

Conditions: see Fig. 4C.

Peak	Retention time (min)	
	DryLab	Actual
1	10.3	11.0
2	11.2	11.9
3	20.1	20.6
4	20.8	21.3

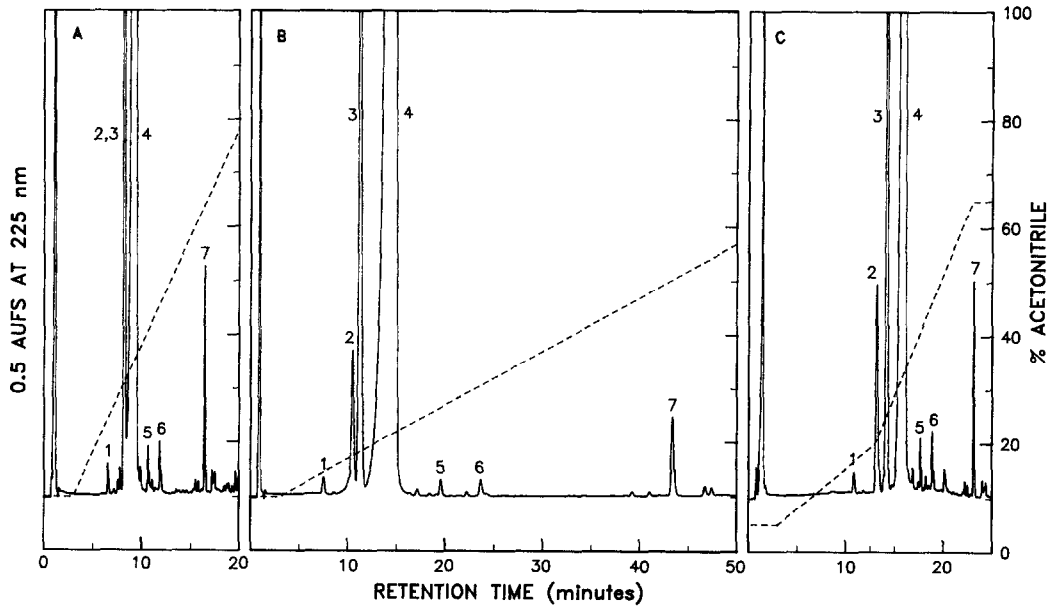

Fig. 3. (A, B) Results of initial gradient experiments run to acquire data for DryLab G/plus for 2-methylcarboxybenzaldehyde (peak 4). Mobile phase: water–acetonitrile. Column: 15 × 0.46 cm I.D. Spherisorb C_8, 5 μm. Flow: 1.5 ml/min. Gradient: 10 to 90% acetonitrile in water in 20 min (A) and in 80 min (B). (C) Optimized gradient separation based upon DryLab G/plus predictions. Gradient: 5 to 20% acetonitrile in 10 min then to 65% acetonitrile by 20 min. Sample: 10 μl of 2-methylcarboxybenzaldehyde at 10 mg/ml in acetonitrile. Peaks: 1–3 = impurities; 4 = 2MCBA; 5–7 = impurities.

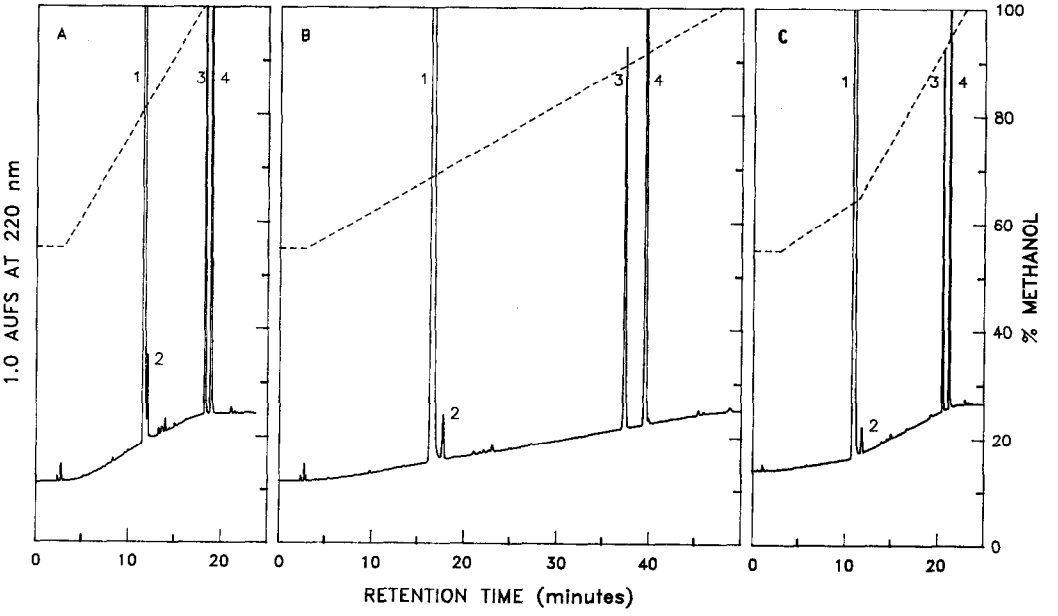

Fig. 4. (A, B) Results of initial gradient experiments run to acquire data for DryLab G/plus for cyclooctatetraene (COT, peak 1). Mobile phase: water–methanol. Column: 25 × 0.46 cm I.D. Spherisorb ODS-2, 5 μm cartridge. Flow: 1.25 ml/min. Gradient: 55 to 100% methanol in 15 min (A) and in 45 min (B). (C) Optimized gradient separation based upon DryLab G/plus predictions. Gradient: 55% to 65% methanol in 8.5 min then to 100% methanol from 8.5 to 20 min. Sample: 10 μl of COT at 5 mg/ml in methanol. Peaks: 1 = COT; 2 = unknown impurity; 3, 4 = dimeric impurities.

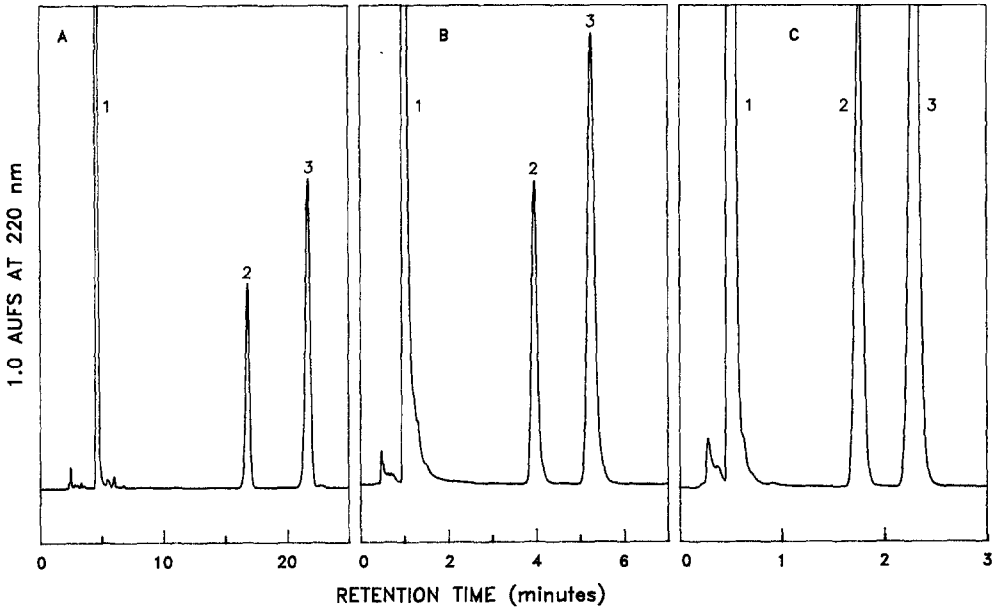

Fig. 5. Optimized analytical scale isocratic separation of dimers from COT based on DryLab I/plus predictions from the gradient separations shown in Fig. 4A and 4B. Mobile phase: methanol–water (83:17). Columns and flow-rates: (A) 25 × 4.6 cm I.D. Spherisorb ODS-2 (5 μm), 1.25 ml/min, (B) 7.5 × 0.46 cm I.D. Ultrasphere ODS (3 μm), 2 ml/min and (C) 3.3 × 0.46 cm I.D. Pecosphere C_{18} (3 μm), 2 ml/min. Sample: 10 μl of COT at 5 mg/ml in methanol. Peaks: 1 = COT; 2, 3 = dimers.

Isocratic optimization

Because a simple method to test only for dimers in COT was desired, computer simulations using the data generated from Fig. 4A and 4B (initial gradient trials) were performed with DryLab I/plus to arrive at suitable isocratic conditions for this assay. A reasonable separation could be performed on the 25 cm column used initially (Fig. 5A) with an optimized mobile phase composition of 83% methanol obtained directly from computer simulations; however, the column optimization functions of DryLab I/plus also suggested that satisfactory separation could be maintained on short, 3 μm particle columns with the same mobile phase composition to greatly reduce analysis time to under 3 min as shown in Figs. 5B and C. A comparison between DryLab I/plus predictions and confirming HPLC experiments is given in Table V. The resolutions be-

TABLE V

COMPARISON OF EXPERIMENTAL RETENTION TIMES WITH DryLab I/plus PREDICTIONS FOR THE OPTIMIZED ISOCRATIC SEPARATIONS OF COT FROM IMPURITIES ON VARIOUS COLUMNS

Conditions: see Fig. 5.

Peak	Retention time (min)					
	Spherisorb ODS-2		Ultrasphere ODS		Pecosphere C_{18}	
	DryLab	Actual	DryLab	Actual	DryLab	Actual
COT	4.2	4.6	0.9	1.0	0.4	0.5
Dimer 1	16.8	16.8	3.2	4.0	1.4	1.8
Dimer 2	21.5	21.7	4.1	5.3	1.8	2.3

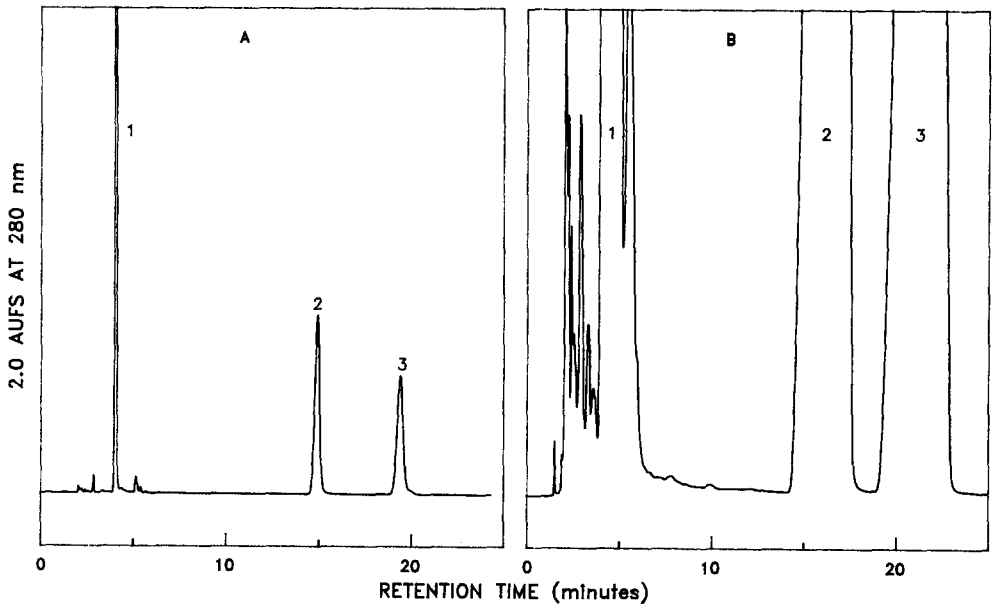

Fig. 6. Optimized semi-preparative isocratic separation of dimers from COT based on DryLab I/plus predictions from the gradient separations shown in Fig. 4A and 4B. Mobile phase: methanol–water (83:17). Column: 25 × 1 cm I.D. Supelco LC-18DB (5 μm) at a flow-rate of 6.0 ml/min. Sample: 2 μl (1.85 mg) of neat COT (A) and 200 μl (185 mg) (B). Peaks: 1 = COT; 2, 3 = dimers.

tween the peaks of interest are large enough to provide for a very rugged separation.

The utility of DryLab I/plus for scaling separations upward was also demonstrated for the separation of COT from dimers. In order to collect fractions of the COT dimer peaks for off-line identification by MS and NMR, the separation was moved to a semi-preparative system, again based directly on the predictions from the initial gradient trials of Fig. 4A and 4B. The results presented in Table VI and Fig. 6 show that the computer optimized separation was accurate and rugged enough to allow for the injection of 200 μl (about 185 mg) of neat COT on a 25 × 1 cm Supelco LC-18DB column at a flow-rate of 6.0 ml/min. It is apparent from Fig. 6B that more than 185 mg of COT could have been loaded onto the semi-preparative column while still maintaining good resolution between the dimer peaks; however the 200-μl injection size was convenient with the WISP autosampler used for the isolation procedure.

TABLE VI

COMPARISON OF EXPERIMENTAL RETENTION TIMES WITH DryLab I/plus PREDICTIONS FOR THE OPTIMIZED SEMI-PREPARATIVE ISOCRATIC SEPARATION OF COT FROM IMPURITIES

Conditions: see Fig. 6A (2-μl injection).

Peak	Retention time (min)	
	DryLab	Actual
1	10.3	11.0
2	11.2	11.9
3	20.1	20.6

DISCUSSION

Overall excellent predictions of retention behavior were observed using DryLab software as an aid in HPLC method development and optimization. Furthermore, many hours of analyst and instrument time and solvents were saved through the use of computer simulations. It is likely that better, more rugged separations were achieved using simulations since time restraints may not have allowed

such a number of "real" experiments and a less optimized solution may have been chosen. To assess their impact on the desired separation, the simulations allow for a wide variety of gradient times, gradient profiles (linear *versus* segmented), column dimensions, particle sizes, flow-rates, etc. to be explored very rapidly and accurately.

Remarkably, separation predictions worked well for COT separations despite the fact that the column packing brands used for experimental HPLC separations were changed several times from that used to acquire initial data for the DryLab program. Indeed, many of the differences in observed *versus* predicted retention are probably due largely to the varying column packing chemistries used in the experiments, and not due to errors in the software algorithms.

REFERENCES

1 J. C. Berridge, *Techniques for the Automated Optimization of HPLC Separations*, Wiley, New York, 1985.
2 L. R. Snyder, J. L. Glajch and J. J. Kirkland, *Practical HPLC Method Development*, Wiley-Interscience, New York, 1988.
3 J. L. Glajch and L. R. Snyder (Editors), *Computer-Assisted Method Development in Chromatography; J. Chromatogr.*, 485 (1989).
4 G. Szepesi, *HPLC in Pharmaceutical Analysis*, Vol. I, CRC Press, Boca Raton, FL, USA, 1990, Ch. 6, p. 101–173.
5 L. R. Snyder, J. W. Dolan and D. C. Lommen, *J. Chromatogr.*, 485 (1989) 65.
6 J. W. Dolan, D. C. Lommen and L. R. Snyder, *J. Chromatogr.*, 485 (1989) 91.