Journal of Chromatography, 302 (1984) 135–147
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 435

COMPUTER SIMULATION AS AN AID TO OPTIMIZING CONTINUOUS-DEVELOPMENT TWO-DIMENSIONAL THIN-LAYER CHROMATOGRAPHY

ERIC K. JOHNSON and DAVID NUROK*
Department of Chemistry, Indiana University-Purdue University at Indianapolis, P.O. Box 647, Indianapolis, IN 46223 (U.S.A.)

SUMMARY

A method for the computer simulation of continuous-development thin-layer chromatography (TLC) for the separation of a mixture of 30 steroids is described. Both normal-phase–normal-phase and normal-phase–reversed-phase two-dimensional TLC are discussed. Examples are presented illustrating the agreement between predicted and experimental chromatograms.

INTRODUCTION

One of the advantages of thin-layer chromatography (TLC) is that it can be performed in the two-dimensional mode. This allows two solvent systems of totally different selectivity to be combined in a sequential and synergistic manner, to effect difficult separations. The technique has the potential for separating more complex mixtures than is currently possible with high-performance liquid chromatography, as has been pointed out in a recent comprehensive review by Zakaria *et al.*[1]. In spite of this potential, two-dimensional TLC is not very widely used. One of the reasons for this is that it is difficult to predict the experimental conditions that yield the optimum separation of a complex mixture by two-dimensional TLC, even though computer simulation as an aid to optimization shows promise[2,3].

It is necessary to choose solvents of different selectivity in order to fully utilize the separation power of two-dimensional TLC. If the same solvent is used for both developments the spots will be distributed along the diagonal that stretches from the origin to the opposite corner of the plate. In this case no real advantage is gained over conventional TLC. A very good choice of solvent systems for each of the two developments would result in spots being evenly distributed across the entire area of the TLC plate, while an excellent choice of solvent systems would have a higher concentration of spots near the origin and fewer spots distant from the origin to allow for the spot spreading that occurs as solutes migrate. Such choice of solvents can be a formidable task, specially when performed by trial and error. Nevertheless some very good separations have been performed by two-dimensional TLC, as illustrated in the review by Zakaria *et al.*[1].

0021-9673/84/$03.00

One approach to optimizing this technique is to calculate which combination of solvent systems would give the most satisfactory separation. Gonnord *et al.*[2] have shown that this can be done by having a computer program compare the distances between all pairs of spots for any given combination of solvent systems. These authors used two equations, the first of which gives equal weighing to all spot pairs and the second of which gives more weight to poorly resolved pairs. The second equation predicted which combination of two solvent systems selected from a list of ten systems reported for 2,4-dinitrophenyl amino acids would give a good separation with all the spots distributed over a large area of the plate.

We report on a different approach to computer-aided optimization of two-dimensional TLC with binary solvent systems and continuous development for the separation of a mixture of 30 steroids.

EXPERIMENTAL

The steroids used were purchased from Sigma (St. Louis, MO, U.S.A.) and the solvents used were purchased from Aldrich (Milwaukee, WI, U.S.A.).

The Regis SB/CD chamber was used for chromatography. The use of this chamber for optimized continuous-development TLC has recently been described[4]. The chamber is used in a laboratory hood with the front sash lowered sufficiently to allow efficient evaporation of solvent to occur from the protruding edge of the plate. In our laboratory, this corresponds to a gap of 0 to 4 in. from the bottom of the sash to the top of the chamber, depending on the volatility of the solvent used.

The TLC plates were a gift from Whatman Chemical Separation Inc. (Clifton, NJ, U.S.A.). Two-dimensional TLC was performed on K5 silica gel plates, catalogue No. 4850-820, and SC5 Multi-K (dual-phase) plates, catalogue No. 4804-800. Preliminary data were obtained on either K5 or KC18 plates, catalogue No. 4801-800. The K5 silica gel plates and KC18 reversed-phase plates were cut into 10 × 10 cm sections before use. The SC5 plates, which consist of a 20 × 3 cm silica gel layer, contiguous with a 20 × 17 cm C_{18}-layer, were used as follows.

The plate was cut to 14.4 × 10 cm with the silica gel area being 3 × 10 cm. The sample was spotted 1 cm from the bottom edge and 1.5 cm from the side edge within the silica gel strip. The plate was placed in position 5 of the SB/CD chamber, which results in 8.3 cm of the TLC plate being in the chamber and 1.7 cm protruding as a solvent evaporation area. At the end of the first development, which was with normal-phase solvent, the plate was removed from the tank and placed in a laboratory hood for sufficient time to remove the solvent.

The second development was performed with the plate rotated 90° from its original position *i.e.* with the 10 cm edge of the silica strip contacting the bottom of the chamber. Position 3 of the SB/CD chamber was used, where 5.4 cm of the plate is in the chamber. The plate was developed with acetone as solvent in the conventional (non-continuous development) manner until the solvent front reached the cover plate *i.e.* with a solvent path length of 5.4 cm. The spots all migrate with the solvent front due to the solvent strength of acetone. The acetone is removed and a section of the plate 4.4 × 10 cm, which includes the silica gel strip, is cut from the main body of the plate and discarded. The above procedures result in a 10 × 10 cm C_{18} TLC plate with the solutes deposited along a line 10 mm from the edge.

This plate is then developed in position 5 of the SB/CD chamber with the reversed-phase solvent.

The K5 and SC5 plates were maintained at a relative humidity of 60% until immediately before use by storage over 39% sulfuric acid in a desiccator.

The steroids were visualized as described previously[5].

RESULTS AND DISCUSSION

The two-dimensional separation of steroids by continuous development TLC with binary solvent systems allows great flexibility in choosing conditions for separating a complex mixture. There are six variables that must be optimized when this technique is used: the solvent path length, the concentration of the binary components and the development time, in each dimension. The additional variables result in a system more complex to optimize than that described by Gonnord *et al.*[2]. It should be noted that in the analogous single-dimension TLC, there are only three variables, and the optimization procedure is straightforward[6]. However, it does not necessarily follow that the optimum conditions for two-dimensional TLC are simply the combination of the conditions for two optimized one-dimensional separations.

There are various approaches to optimizing continuous-development two-dimensional TLC. A rigorous approach would be to choose a separation parameter, such as one of the two defined by Gonnord *et al.*[2], and use this as the dependent variable with the simplex optimization procedure. A less rigorous approach would be to use fixed solvent path lengths in each dimension, to work in terms of spot separation for individual pairs of compounds, and to use the overlapping resolution map (ORM) procedure[7]. Spot separation for two-dimensional continuous development TLC has already been defined[3], and the ORM technique has already been described for single-dimensional TLC[8]. The variables would be reduced to three to correspond to the triangular axes. These would be mole fraction in each dimension and development time in one of the two dimensions. The development time in the second dimension would be a dependent variable, defined as the time for the spot with highest R_F to reach the end of the plate. Fixed plate lengths are justifiable, because complex mixtures will often require a large fraction of the longest solvent path compatible with efficient chromatography, *i.e.* about 10 cm for modern TLC plates. Fixing these variables would not cause inferior separation but could result in longer analysis time than necessary for those mixtures that require a path length shorter than the defined length.

A variation of the above approach would be to construct a three-dimensional window diagram with solvent composition in each dimension as the two independent variables. Plate lengths would be fixed and development time in both dimensions would be a dependent variable, defined as for the ORM case described above. While this approach would be useful, it could result in significantly longer analysis times than necessary.

The approach that we have used in this paper is less rigorous than any of the above techniques. Two forms of two-dimensional TLC were performed with the 30 steroids listed in Table I. In the first form the development in both dimensions was on a silica gel plate, whereas in the second form a dual-phase plate was used, which allows the first development on silica gel and the second development on a C_{18}-layer.

TABLE I

LIST OF STEROIDS CHROMATOGRAPHED

No.	*Trivial name*	*Systematic name*
1	17α-Acetoxyprogesterone	17α-Acetoxy-4-pregnene-3,20-dione
2	Androstanedione	5α-Androstane-3,17-dione
3	Androstanolone	17β-Hydroxy-5α-androstan-3-one
4	Androstenediol	5-Androstene-3β.17β-diol
5	Androstenedione	4-Androstene-3,17-dione
6	Androsterone	3α-Hydroxy-5α-androstan-17-one
7	Betamethasone	9α-Fluoro-11β,17,21-trihydroxy-16β-methyl-1,4-pregna-diene-3,20-dione
8	Cholesterol	5-Cholesten-3β-ol
9	Cortisone	17α,21-Dihydroxy-4-pregnene-3,11,20-trione
10	Corticosterone	11β,21-Dihydroxy-4-pregnene-3,20-dione
11	7-Dehydrocholesterol	5,7-Cholestadien-3β-ol
12	Dexamethasone	9α-fluoro-11β,17,21-Trihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione
13	Diethylstilbesterol	3,4-Bis(*p*-hydroxyphenyl)-3-hexene
14	Epiandrosterone	3β-Hydroxy-5α-androstan-17-one
15	Ergosterol	Ergosta-5,7,22-triene-3β-ol
16	17β-Estradiol	Estra-1,3,5(10)-triene-3,17β-diol
17	Estrone	3-Hydroxy-1,3,5(10)estratrien-17-one
18	Ethisterone	17α-Ethynyl-17β-hydroxy-4-androsten-3-one
19	Hydrocortisone	11β,17α,21-Trihydroxy-4-pregnene-3,20-dione
20	Lanosterol	8,24-Lanostadien-3β-ol
21	Mestranol	17α-Ethynyl-3-methoxy-1,3,5(10)-estratrien-17β-ol
22	Methandriol	17α-Methyl-5-androstene-3β,17β-diol
23	Prednisone	17α,21-Dihydroxy-1,4-pregnadiene-3,11,20-trione
24	Pregnanediol	5α-Pregnane-3β,20β-diol
25	Pregnenolone	3β-Hydroxy-5-pregnen-20-one
26	11-Deoxy-17-hydrocorticosterone	17,21-Dihydroxy-4-pregnene-3,20-dione
27	Spironolactone	7α-(Acetylthio)-17α-hydroxy-3-oxo-4-pregnene-21 carboxylic acid γ-lactone
28	Stigmasterol	24-Ethyl-5,22-cholestadien-3β-ol
29	Testosterone	17β-Hydroxy-4-androstene-3-one
30	Tomatidine	5α-Tomatidan-3β-ol

The retention data for these steroids are listed in Table II as slopes and intercepts in the following equation:

$$\ln k = a \ln X_s + b \tag{1}$$

where k is the capacity factor and X_s is the mole fraction of the strong solvent in the binary system.

The solvents used for silica gel chromatography were ethyl formate, butyl acetate, nitromethane and tetrahydrofuran. Each was used as a binary mixture with toluene. The solvent for reversed-phase chromatography was acetonitrile–0.5 *M* sodium chloride.

It has been shown[3] that the distance migrated by a given solute in continuous-development TLC with a binary solvent system is given by:

$$M_D = \frac{1}{1 + \exp(a \ln X_s + b)} \left[\frac{l^2 - 2lx + \kappa t_l}{2l} \right] \tag{2}$$

where M_D is the distance migrated, a, b and X_s are defined as in eqn. 1, l is the solvent path length used for continuous development, x is the distance from solvent origin to spot origin, κ is the solvent velocity constant, and t_l is the development time.

It is necessary to experimentally determine the values of a, b and κ for each solute in each binary system. The constant κ can be expressed as a function of X_s using a second order polynomial[8]. Plots of κ *vs.* X_s are shown in Fig. 1 for the solvents used.

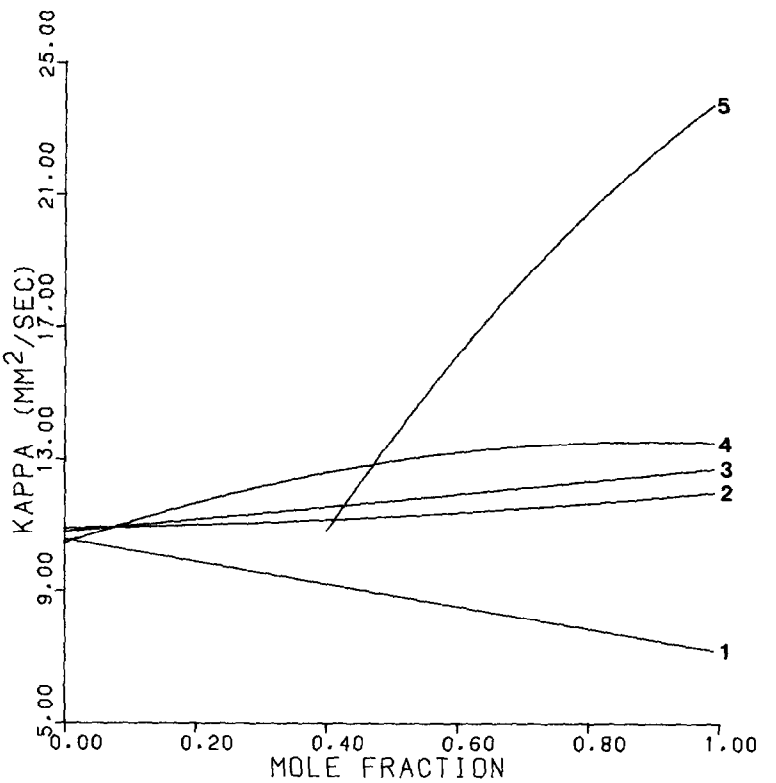

Fig. 1. Plots of κ *vs.* mole fraction for five solvents: butyl acetate (1), nitromethane (2), tetrahydrofuran (3), ethyl formate (4), and acetonitrile (5). Solvents 1–4 were chromatographed on silica gel as binary systems with toluene. Acetonitrile was chromatographed on a C_{18} reversed-phase layer as a binary with 0.5 *M* sodium chloride. TLC plate length for all measurements was 8.3 cm.

The strategy used for the separation of the 30 steroids, listed in Table I, by two-dimensional TLC is as follows. Eqn. 2 was used to construct M_D *vs.* X_s plots for all the solutes in each of the binaries studied. The values of a, b and κ were determined experimentally. The value of l was specified at 8.3 cm and the value of t_l was so chosen for each plot that the compound with the highest R_F migrates to the end of the plate. Two of the plots are shown in Figs. 2 and 3. Both the overall shape and the selectivity shown for different pairs of compounds vary with the identity of the binary system. However, in all cases migration distance and selectivity change with increasing mole fraction of the binary.

TABLE II

RETENTION DATA FOR 30 STEROIDS GIVEN AS SLOPES AND INTERCEPTS IN EQN. 1

*Steroid**	*Tetrahydrofuran***		*Butyl acetate***		*Nitromethane***		*Ethyl formate***		*Acetonitrile****	
	Slope	*Intercept*	*Slope*	*Intercept*	*Slope*	*Intercept*	*Slope*	*Intercept*	*Slope*	*Intercept*
1	−1.37	−2.87	−1.18	−1.17	−2.06	−1.83	−4.08	−1.79	−1.57	−0.82
2	−1.09	−2.97	−0.94	−1.79	−1.67	−2.15	−0.61	−1.92	−1.27	−0.70
3	−1.19	−2.30	−1.02	−1.15	−1.59	−1.47	−1.12	−1.55	−0.80	−0.30
4	−1.29	−1.81	−1.14	−0.67	−1.67	−0.96	−1.56	−1.16	−0.74	−0.56
5	−1.15	−2.52	−0.86	−0.94	−1.87	−1.73	−1.18	−1.68	−1.30	−1.03
6	−1.34	−2.49	−1.23	−1.19	−1.78	−1.35	−1.23	−1.52	−0.96	−0.33
7	−2.83	−2.19	−2.33	0.39	−3.34	0.88	−3.59	−0.59	−1.11	−2.59
8	−0.94	−2.18	−0.93	−1.72	−1.66	−2.45	−1.41	−2.12	−2.46	3.02
9	−2.47	−2.01	−1.22	0.93	−3.72	−0.01	−3.19	−0.66	−2.31	−3.84
10	−2.27	−1.83	−1.41	1.07	−2.60	0.32	−2.51	−0.44	−0.54	−1.47
11	−0.95	−2.18	−2.58	−2.69	−1.51	−2.13	−0.66	−1.86	−2.85	0.96
12	−2.75	−1.96	−2.26	0.64	−3.33	0.90	−3.61	−0.54	−0.83	−2.39
13	−0.79	−1.95	−0.95	−2.45	−1.73	−2.22	−1.27	−2.09	−1.69	−2.02
14	−1.12	−1.97	−1.02	−0.95	−1.54	−1.26	−1.09	−1.37	−0.73	−0.23
15	−0.96	−2.14	−0.97	−1.64	−1.48	−2.12	−1.41	−2.08	−5.35	1.13
16	−1.26	−2.23	−1.10	−1.44	−1.86	−1.42	−1.69	−1.70	−1.25	−1.65
17	−1.02	−2.59	−1.19	−2.40	−1.83	−2.15	−1.60	−2.30	−1.61	1.84
18	−1.30	−2.79	−1.27	−1.65	−1.73	−1.57	−0.91	−1.68	−1.04	−0.98
19	−3.29	−2.64	−2.04	1.15	−2.87	1.33	−3.21	−0.21	−1.81	−3.50
20	−0.91	−2.73	−1.07	−2.63	−1.58	−3.01	−0.64	−2.34	−5.69	2.07
21	−0.98	−3.02	−1.06	−2.85	−1.67	−2.88	−1.32	−2.61	−1.78	−0.95
22	−1.13	−1.68	−1.11	−0.86	−1.44	−0.79	−1.27	−1.09	−0.37	0.08
23	−2.34	−1.70	−1.93	1.10	−3.79	0.50	−2.94	−0.32	−0.48	−2.34
24	−1.38	−1.98	−1.13	−0.75	−1.81	−1.09	−1.65	−1.36	−1.06	0.30
25	−1.05	−2.06	−0.88	−1.16	−1.64	−1.71	−1.20	−1.57	−1.19	−0.05
26	−2.61	−2.37	−1.15	0.10	−2.34	0.02	−2.32	−0.84	−0.52	−1.39
27	−1.50	−2.80	−1.32	−0.88	−2.05	−1.59	−1.19	−1.66	−1.47	−1.51
28	−0.96	−2.28	−1.00	−1.76	−1.53	−2.26	−0.93	−1.86	−8.05	2.48
29	−1.45	−2.22	−1.27	−0.50	−1.95	−1.13	−1.79	−1.37	−1.66	−1.01
30	−1.98	−0.96	−1.35	0.88	−1.52	−2.99	−1.27	0.91	−0.32	−0.04

* See Table I for steroid assignments.

** Solvent diluted with toluene and chromatography performed on silica gel.

*** Solvent diluted with 0.5 *M* sodium chloride and chromatography performed on a C_{18}-layer.

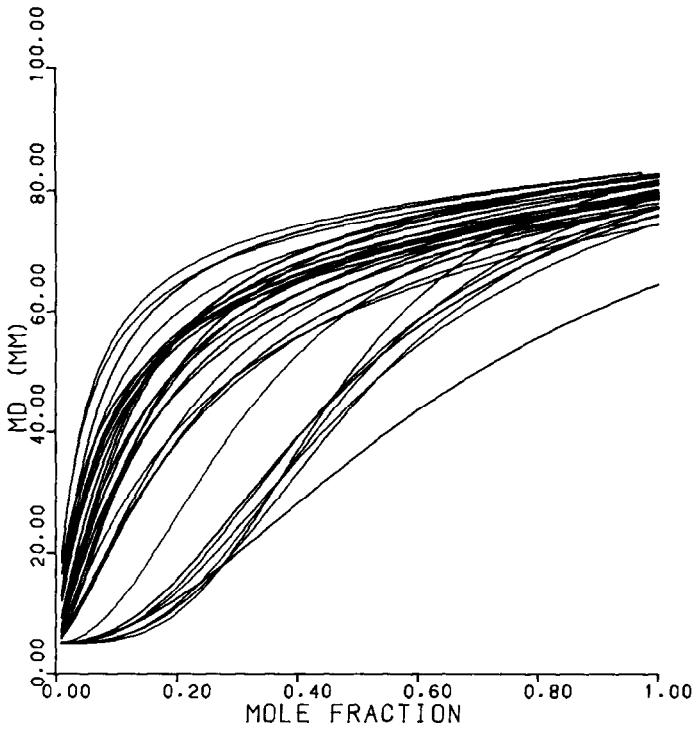

Fig. 2. Plots of computed spot migration *vs.* mole fraction for 30 steroids chromatographed on a silica gel plate with tetrahydrofuran–toluene as solvent. Plate length is 8.3 cm and development time is 665 sec.

It is a formidable task to decide by inspection alone which combination of two binaries, and at what mole fraction of each, would give a good separation of a complex mixture by two-dimensional TLC. However it is possible to tell by inspection which mole fractions afford relatively good selectivity within a particular binary, even though there is always overlap of some compounds at any mole fraction in a sufficiently complex mixture. The strategy used was as follows: three mole fractions at which a large number of compounds can be separated are chosen for each binary by inspection of the M_D *vs.* X_s plot. For the system tetrahydrofuran–toluene (Fig. 2) the mole fractions chosen are 0.1, 0.2, and 0.3; for the binary nitromethane–toluene (Fig. 3) the mole fractions are 0.3, 0.5 and 0.8. The four solvent systems studied can be paired in six combinations for two-dimensional TLC. Within each of these pairs the binary systems can be combined in nine different ways by considering all the possible combinations of the three chosen mole fractions.

The nine computer-generated chromatograms for the solvent system (nitromethane–toluene)–(tetrahydrofuran–toluene) are shown in Fig. 4. The method of generating such plots has been described previously[3]. The development time in each dimension is so chosen that the compound with the highest R_F migrates nearly to the end of the plate. Fig. 4 represents one of the poorest of the six possible combinations of normal phase solvent systems considered in this study. The compounds are distributed over a small elongated area of the plate that is approximately parallel

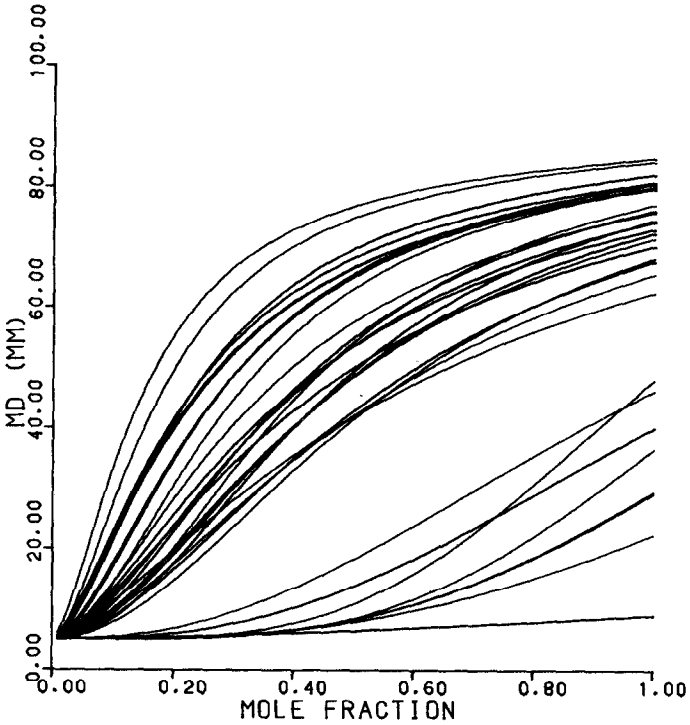

Fig. 3. Plots of computed spot migration *vs.* mole fraction for 30 steroids chromatographed on a silica gel plate with nitromethane–toluene as solvent. Plate length is 8.3 cm and development time is 760 sec.

to the 45° diagonal. Thus, this combination of binaries somewhat resembles a two-dimensional chromatogram with the same solvent system for both developments.

Fig. 5 shows the computer generated chromatograms for the system (butyl acetate–toluene)–(ethyl formate–toluene). The spots are more widely scattered than in Fig. 4, even though the spots are still confined to a relatively small area in many of the chromatograms. Nevertheless, the top left chromatogram shows the spots relatively well scattered over about 50% of the area of the plate. Fig. 6 shows that reasonable agreement can be obtained between predicted and experimental chromatograms.

There is a group of seven compounds that remain near the origin in Fig. 6. These compounds can be separated under a different set of experimental conditions that are shown in Fig. 7. For this particular chromatogram there is a good agreement between predicted and experimental chromatograms. The compounds with the higher R_F migrate to the end of the plate in each dimension and are present as a conglomerate spot on the top right hand corner of the plate. The size of the spots in Fig. 7 are considerably larger than in the other chromatograms reported. This may be due to the long development time, which is 150 min in each dimension.

Care must be taken to perform the two-dimensional TLC under the same experimental conditions as were used for collecting the data on which the computer generated chromatograms are based. It is essential to store the plates at a defined

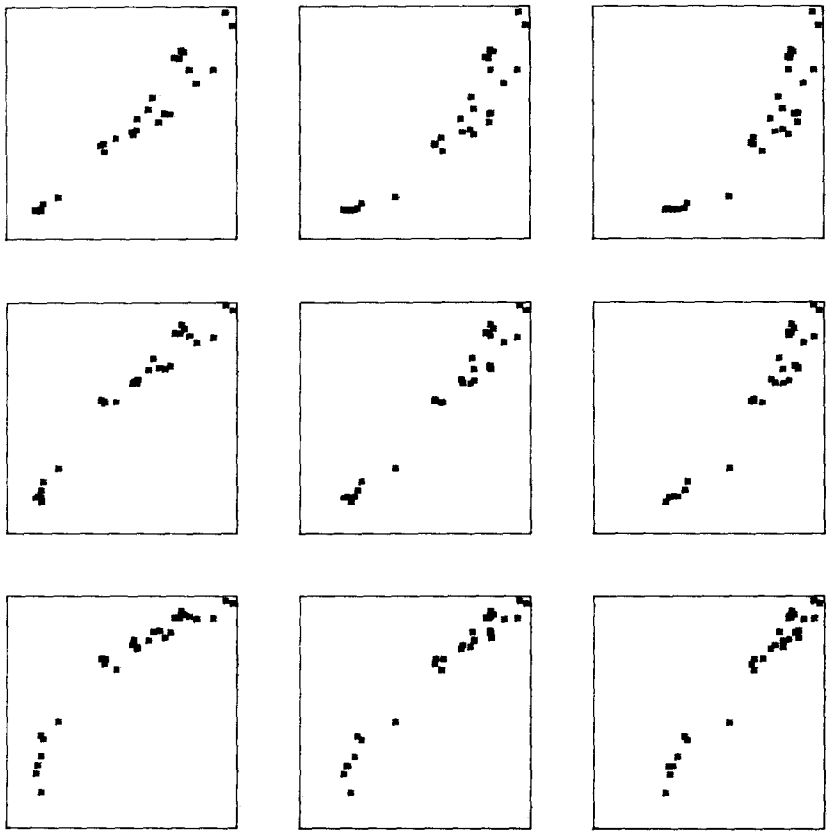

Fig. 4. Computer-simulated two-dimensional chromatograms for the system nitromethane–toluene (vertical axis) and tetrahydrofuran–toluene (horizontal axis) on silica gel plates. The origin of each chromatogram is at the bottom left hand corner. Read the above diagram as a matrix with the mole fraction (development time in sec) for the solvent system nitromethane–toluene being 0.3 (960), 0.5 (770) and 0.8 (650) for the top, middle and bottom chromatograms; and for the solvent system tetrahydrofuran–toluene being 0.1 (1130), 0.2 (860) and 0.3 (760) for the left, middle and right chromatograms.

humidity until immediately before use. We have found some difficulty in repeating chromatograms after the lapse of several weeks. This could be due to fluctuations in ambient humidity, temperature or barometric pressure or due to the method in which the plates are stored. These factors are currently being investigated.

The second development in two-dimensional TLC occurs on a plate that has been developed with another solvent system. This could result in migration distances being different from those obtained on a plate that had not been so treated. Preliminary experiments with (butyl acetate–toluene)–(tetrahydrofuran–toluene) show that there are small differences between the unused and the pre-developed plates but that these differences are of the same magnitude as those found between replicate runs on unused plates. The preliminary data for all the chromatograms reported were obtained on plates that had not been prewashed with a solvent. Nevertheless, care should be exercised in this respect when working with untried solvent systems.

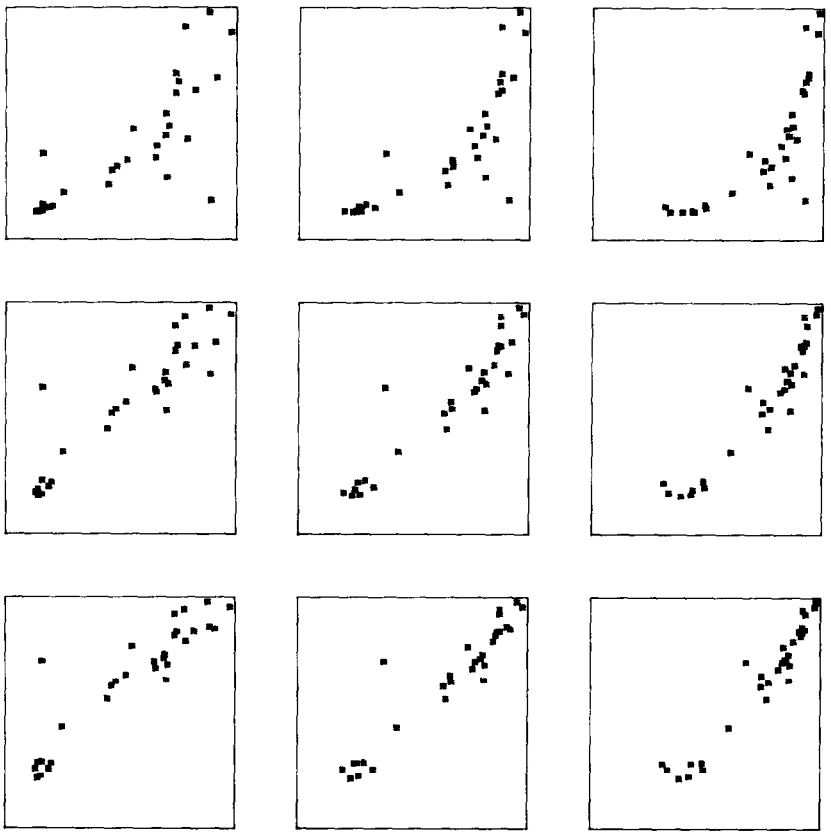

Fig. 5. Computer-simulated two-dimensional chromatograms for the system butyl acetate–toluene (vertical axis) and ethyl formate–toluene (horizontal axis) on silica gel plates. The origin of each chromatogram is at the bottom left hand corner. Read the above diagram as a matrix with the mole fraction (development time in sec) for the solvent system butyl acetate–toluene being 0.1 (1430), 0.4 (930) and 0.6 (930) for the top, middle and bottom chromatograms; and for the solvent system ethyl formate–toluene being 0.3 (760), 0.5 (660) and 0.7 (610) for the left, middle and right chromatograms.

Good separations are achieved by two-dimensional TLC when the selectivities of each development are significantly different. As shown above, this can be difficult to achieve when both developments are in the normal-phase mode. It can however be conveniently achieved when using a dual-phase TLC plate where the first development is performed by normal-phase TLC on a silica gel layer and the second development is performed in the reversed-phase mode on a C_{18}-layer. It is most convenient to perform the developments in this order, because the silica gel layer requires storage at a defined humidity until immediately before TLC. If the reversed phase development was performed first, a lengthy equilibration period would be required after the removal of the aqueous solvent to allow the silica gel layer to equilibrate at the defined humidity. Moreover it is easier to remove the non-aqueous normal-phase solvent than the aqueous reversed-phase solvent. Dual-phase TLC

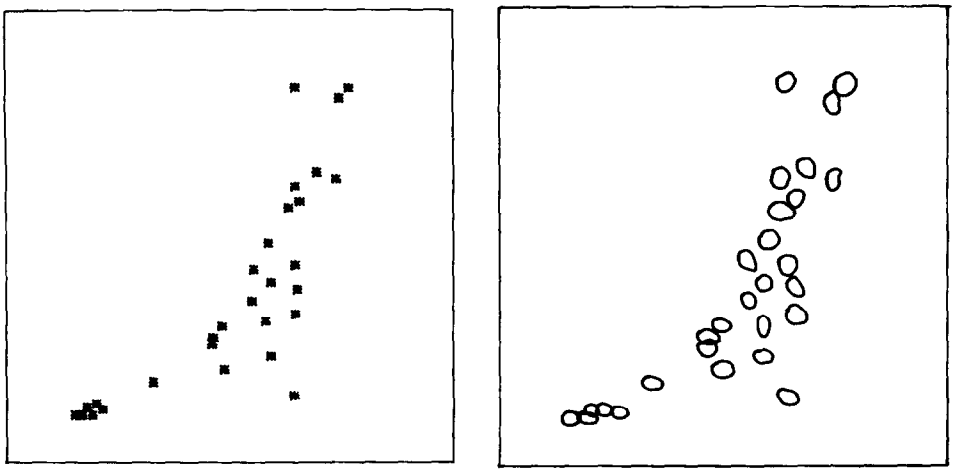

Fig. 6. Computer-simulated (left) and experimental (right) chromatograms for two-dimensional chromatography performed on a silica gel plate. The vertical axis is a butyl acetate–toluene development (mole fraction 0.1, development time 1430 sec), the horizontal axis is a tetrahydrofuran–toluene development (mole fraction 0.2, development time 860 sec). The solvent path length in each dimension is 8.3 cm. The above diagrams are for a 10 × 10 cm plate which includes the solvent evaporation area.

plates, with a narrow silica gel layer and a wide C_{18}-layer are commercially available and were used in this study.

It is necessary to follow the procedure described in the experimental section where the spots are transferred to the C_{18}-layer before commencing the second development. This removes the complication of having silica gel as part of the path for this development.

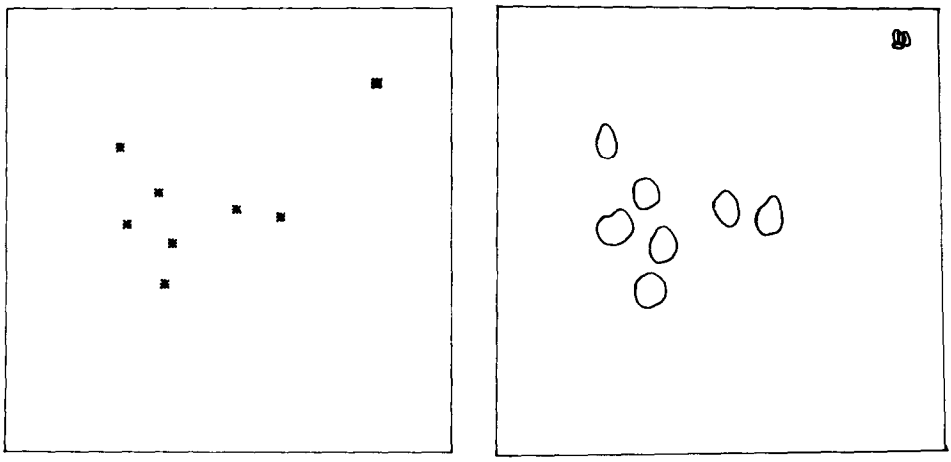

Fig. 7. Computer-simulated (left) and experimental (right) chromatograms for two-dimensional chromatography performed on a silica gel plate. The vertical axis is a butyl acetate–toluene development (mole fraction 0.6, development time 9000 sec), the horizontal axis is a tetrahydrofuran–toluene development (mole fraction 0.2, development time 9000 sec). The dimensions of the diagram are as in Fig. 6.

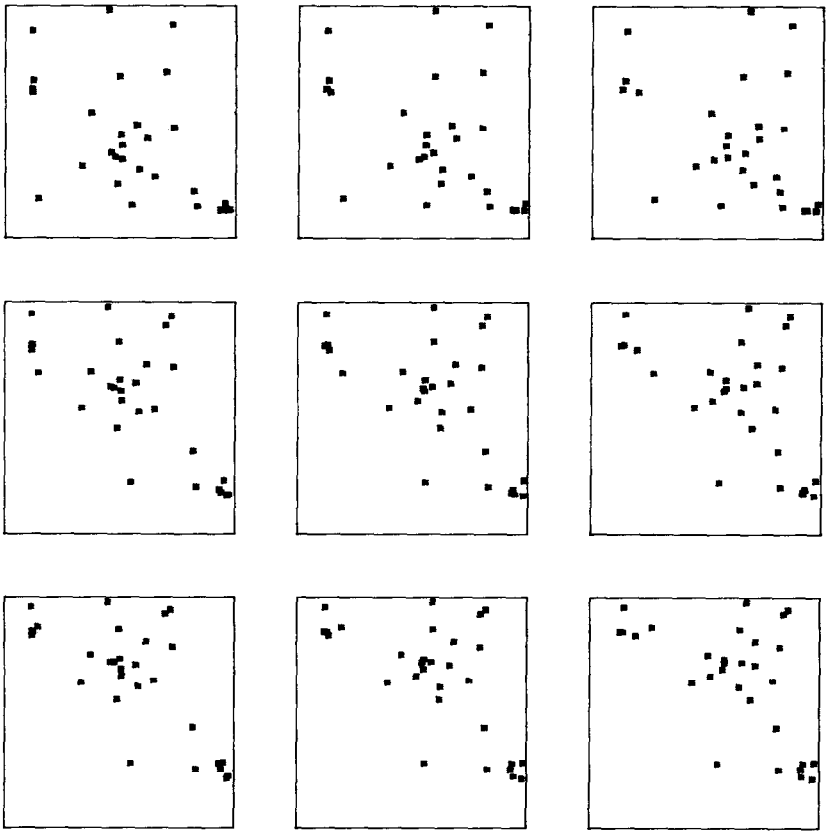

Fig. 8. Computer-simulated two-dimensional chromatograms for the system butyl acetate–toluene (vertical axis) and acetonitrile–aqueous 0.5 *M* sodium chloride (horizontal axis) on dual phase TLC plates. The origin of each chromatogram is at the bottom left hand corner. Read above diagram as a matrix with the mole fraction (development time in sec) for the system butyl acetate–toluene on a silica gel layer being 0.1 (430), 0.4 (930) and 0.6 (930) for the top, middle and bottom chromatograms; and for the system acetonitrile–aqueous 0.5 *M* sodium chloride on a reversed-phase, C_{18}-layer being 0.4 (780), 0.6 (475) and 0.8 (350) for the left, middle and right chromatograms.

Aqueous acetonitrile was chosen as the solvent for preliminary study of dual-phase, two-dimensional TLC. The solvent was prepared from a 0.5 *M* sodium chloride solution in order to stabilize the reversed-phase layer. In spite of this precaution, the solvent front becomes uneven during continuous development when the mole fraction of acetonitrile drops below 0.4. Mole fractions of 0.4, 0.6 and 0.8 were chosen for pairing this binary system with each of the four normal-phase binaries. Fig. 8 shows the set of computer-generated chromatograms for the system (butyl acetate–toluene)–aqueous acetonitrile. This is the best of the systems that were considered. In the top three chromatograms the solute spots are well distributed over most of the plate area. Fig. 9 shows that good agreement is obtained between the computer predicted and the experimental chromatograms.

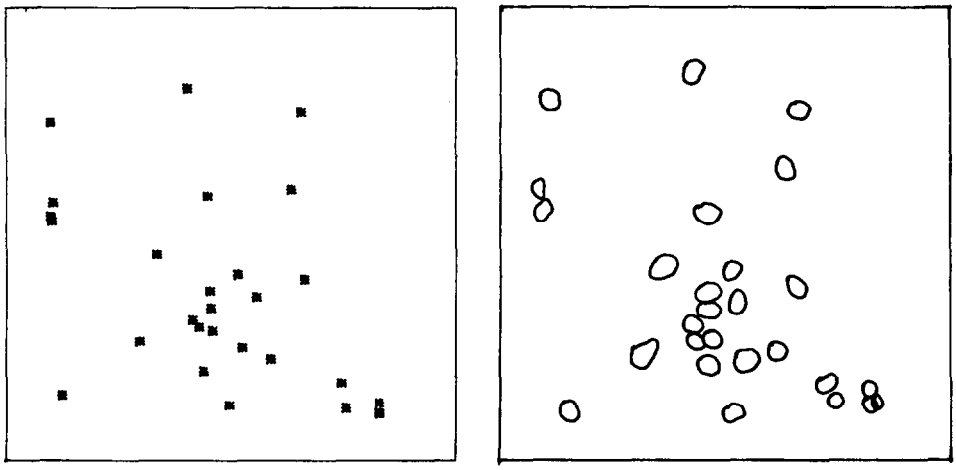

Fig. 9. Computer-simulated (left) and experimental (right) chromatograms for two-dimensional chromatography performed on a dual-phase plate. The vertical axis is a butyl acetate–toluene development (mole fraction 0.1, development time 1430 sec) on silica gel, the horizontal axis is an acetonitrile–aqueous 0.5 *M* sodium chloride development (mole fraction 0.4, development time 900 sec) on a C_{18}-layer. The dimensions of the diagram are as in Fig. 6.

CONCLUSION

Computer simulation of continuous-development two-dimensional TLC is a useful tool for choosing experimental conditions. It allows the rapid evaluation of a large number of solvent systems, once the preliminary data are collected. The best separation is obtained when the selectivities of the two TLC systems are significantly different. This is relatively difficult to accomplish for the steroid mixture considered above, when both developments are on a silica gel layer. However, a good separation is obtained when a dual-phase plate is used which allows the first development to be performed on a silica gel layer and the second on a C_{18}-layer.

ACKNOWLEDGEMENTS

This work was supported by a grant from Dow Chemical. The TLC plates used were a gift of Whatman Chemical Separation Inc.

REFERENCES

1 M. Zakaria, M.-F. Gonnord and G. Guiochon, *J. Chromatogr.*, 271 (1983) 127.
2 M.-F. Gonnord, F. Levi and G. Guiochon, *J. Chromatogr.*, 264 (1983) 1.
3 D. Nurok, R. E. Tecklenburg, Jr. and B. L. Maidak, *Anal. Chem.*, 56 (1984) 293.
4 R. E. Tecklenburg, Jr., E. K. Johnson and D. Nurok, *Anal. Chem.*, 55 (1983) 2196.
5 D. Nurok, R. M. Becker and K. A. Sassic, *Anal. Chem.*, 54 (1982) 1955.
6 R. E. Tecklenburg, Jr., B. L. Maidak and D. Nurok, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 627.
7 J. L. Glajch, J. J. Kirkland, K. M. Squire and J. M. Minor, *J. Chromatogr.*, 199 (1980) 57.
8 R. E. Tecklenburg, Jr., G. H. Fricke and D. Nurok, *J. Chromatogr.*, 290 (1984) 75.