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Short Communication

Analysis of plant extracts by multiple development thin-layer chromatography

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ABSTRACT

The simplest version of multiple gradient development, two-stage development, is discussed. A computer program for calculation of final R_F values in this gradient mode for known retention *vs.* eluent composition relationships for different plant extracts was used. Comparison of predicted and experimental R_F values showed satisfactory agreement.

INTRODUCTION

The analysis of complex samples is a frequent problem in the development of chromatographic methods: the number of components in many samples, *e.g.*, plant extracts, is unknown and may be as high as several hundred. HPTLC is often coupled with other chromatographic techniques as a preliminary step before the main analysis can be done. The properties of the adsorbents used in HPTLC are well known and, combined with broad range of eluents, give good prospects for the analysis of complex samples.

For a mixture with a wide polarity range, it is unlikely that a gradient of mobile phase concentration will provide a complete separation of all sample components. A different kind of gradient can be performed with the use of a sandwich chamber. One variation of this process, called incremental multiple development, can be applied [1]. Decreasing solvent strength gradients are very effective for simpler mixtures where a lower separation capacity can be employed adequately for the separation. A unique feature of incremental multiple development HPTLC is the spot reconcentration mechanism.

In multiple development, the zone widths are approximately constant after the first three or four developments [2]. Some simple guidelines for optimizing multiple development chromatography have been proposed [3]. No systematic investigations connected with an adequate mathematical model were reported until recently. In a previous paper [4], a simple model for two-stage development was proposed, later extended to multi-stage development [5]. The models [4,5] have been combined with computer

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programs that enable one to investigate the influence of various parameters on the final R_F value and, in consequence, the choice of the optimum conditions. In this work the method was verified for several plant extracts employed in therapy.

EXPERIMENTAL

A horizontal DS-type sandwich chamber [6] (Chromdes, Lublin, Poland) adapted to stepwise gradient elution was used. Precoated 50 × 100 mm glass plates for HPTLC (silica gel Si 60; Merck, Darmstadt, Germany) were applied. In isocratic development, an eluent of given composition was introduced into the reservoir. The samples were spotted on a dry layer of adsorbent as narrow strips. The solvent flow was observed and stopped after reaching the end line (distance *ca.* 80 mm). The components of the sample were detected by irradiation with a UV lamp. Calculations were performed on an IBM 486 computer with programs presented earlier [4].

Solutions of investigated mixtures (0.01%) were prepared by drying aliquots of the preparations, extracting with ethyl acetate, filtering, evaporating the extract and dissolving the residue in the eluent. The following mixtures were investigated: Cholesol (Herbapol, Wrocław, Poland), containing Extr. Cortex Frangulae, Herba Equiseti, Fructus Rosae, Anthodium Chamomillae, Fr. Coriandri, Fr. Juniperi, Herba Polygoni avic, Infl. Helichrysi, Herba Hyperici and Intr. Taraxaci; and Seboren (Herbapol), containing Extr. fl. ex Fr. Pastinacae, Rx. Bardanae, Rx. Urticae, Rhiz. Calami and Adiuvans.

In the two-stage mode, the plates were developed for part of the total distance with pure modifier as eluent or with a two-component mobile phase. The plates were dried in air at room temperature for 30 min and developed the full distance with an eluent of lower modifier concentration.

RESULTS AND DISCUSISION

In a series of isocratic developments the parameters of the retention *versus* eluent composition equation were determined by the least-squares method, assuming linear log k' *vs.* log c_{mod} relationships, which follows from the Snyder–Soczewiński competitive adsorption model [7,8]:

$$\log k_{(i,j)} = \log k_{0(j)} - m_{(j)} \log c_{(i)} \quad (1)$$

The parameters (capacity factor $k_{0(j)}$, slope $m_{(j)}$) for the investigated mixture are summarized in Tables I–IV. The data (*i.e.*, slopes and capacity factors) were introduced into the computer program. In the next step, various sets of the gradient program parameters were introduced.

TABLE I

COMPARISON OF PREDICTED ($R_{F(calc.)}$) AND EXPERIMENTAL ($R_{F(exp.)}$) R_F VALUES OF GLYCOSIDES

Two-stage development. System: Silica gel–ethyl acetate + methanol. Development program: $c_{(1)}^{MeOH} = 0.75$, $c_{(2)}^{MeOH} = 0.15$, $z_{(1)} = 0.15$, $z_{(2)} = 1.0$. k_0, m and r are parameters of linear log k *vs.* log c_{MeOH} plots.

No.	Solute	k_0	m	r	$R_{F(calc.)}$	$R_{F(exp.)}$	Error, ΔR_F [a]
1	Lanatoside C	0.025	2.23	0.9605	0.46	0.43	0.03
2	Digitoxin	0.077	0.90	0.9945	0.82	0.80	0.02
3	Digitalin	0.073	2.27	0.9779	0.27	0.30	0.03
4	Desacetyllanatoside C	0.047	2.44	0.9918	0.29	0.33	0.04
5	Strophantin G	0.082	2.31	0.9915	0.24	0.27	0.03
6	Acetyldigitoxin	0.304	2.21	0.9899	0.14	0.20	0.06
7	Convalatoxin	0.061	1.65	0.9899	0.50	0.50	0.00
8	Digoxin	0.023	1.53	0.9954	0.75	0.73	0.02

[a] $\Delta R_F = R_{F(exp.)} - R_{F(calc.)}$.

TABLE II

COMPARISON OF PREDICTED ($R_{F(calc.)}$) AND EXPERIMENTAL ($R_{F(exp.)}$) R_F VALUES OF COMPONENTS OF SEBOREN EXTRACT

Two-stage development. System: silica gel–heptane + diisopropyl ether. Development program: $c_{(1)mod} = 1.0$, $c_{(2)mod} = 0.30$, $z_{(1)} = 0.33$, $z_{(2)} = 1.0$. k_0 and m are parameters of linear log k *vs.* log c_{mod} plots.

No.	k_0	m	$R_{F(calc.)}$	$R_{F(exp.)}$	Error, ΔR_F [a]
1	0.13	0.84	0.73	0.81	0.08
2	0.33	0.63	0.57	0.68	0.11
3	0.31	1.24	0.53	0.55	0.02
4	0.68	1.12	0.48	0.41	0.07
5	0.93	1.03	0.43	0.36	0.07
6	0.80	2.73	0.40	0.20	0.20
7	2.32	1.34	0.32	0.16	0.16
8	3.39	1.65	0.30	0.10	0.20
9	9.06	3.59	0.25	0.03	0.22
10	10.36	2.79	0.01	0.03	0.02
11	0.01	0.72	0.73	0.87	0.02

[a] $\Delta R_F = R_{F(exp.)} - R_{F(calc.)}$.

The variables of the gradient programs were the development distance in the first step, $z_{(1)}$, smaller than 1, and the concentration of modifier in the first, $c_{(1)}$, and second steps, $c_{(2)}$. The development distance in the second step, $z_{(2)}$, was always equal to 1.0. The final R_F values were calculated by computer from the equation derived previously [4]:

$$R_F(j) = z_{(1)}R_{F(1,j)} + [1 - z_{(1)}R_{F(1,j)}]R_{F(2,j)} \quad (2)$$

TABLE III

COMPARISON OF PREDICTED ($R_{F(calc.)}$) AND EXPERIMENTAL ($R_{F(exp.)}$) R_F VALUES OF COMPONENTS OF SEBOREN EXTRACT

Two-stage development. System: silica gel–chloroform + ethyl acetate. Development program: $c_{(1)mod} = 1.0$, $c_{(2)mod} = 0.05$, $z_{(1)} = 0.33$, $z_{(2)} = 1.0$. k_0 and m are parameters of linear log k *vs.* log c_{mod} plots.

No.	k_0	m	$R_{F(calc.)}$	$R_{F(exp.)}$	Error, ΔR_F [a]
1	0.002	1.80	0.80	0.79	0.01
2	0.012	1.24	0.78	0.75	0.03
3	0.027	1.05	0.74	0.70	0.04
4	0.107	0.62	0.71	0.66	0.05
5	0.170	0.58	0.64	0.62	0.02
6	0.240	0.52	0.61	0.59	0.02
7	0.190	0.78	0.52	0.51	0.01
8	0.300	0.72	0.46	0.41	0.05
9	0.560	0.69	0.36	0.32	0.04
10	0.650	0.73	0.32	0.25	0.07
11	0.030	2.91	0.32	0.20	0.12
12	0.202	2.04	0.28	0.16	0.12

[a] $\Delta R_F = R_{F(exp.)} - R_{F(calc.)}$.

TABLE IV

COMPARISON OF PREDICTED ($R_{F(calc.)}$) AND EXPERIMENTAL ($R_{F(exp.)}$) R_F VALUES OF COMPONENTS OF CHOLESOL EXTRACT

Two-stage development. System: silica gel–heptane + diisopropyl ether. Development program: $c_{(1)mod} = 1.0$, $c_{(2)mod} = 0.30$, $z_{(1)} = 0.33$, $z_{(2)} = 1.0$. k_0 and m are parameters of linear log k *vs.* log c_{mod} plots.

No.	k_0	m	$R_{F(calc.)}$	$R_{F(exp.)}$	Error, ΔR_F [a]
1	0.015	2.20	0.88	0.82	0.06
2	0.084	1.59	0.75	0.72	0.03
3	0.149	1.86	0.59	0.60	0.01
4	0.411	1.60	0.44	0.48	0.04
5	0.902	1.18	0.35	0.36	0.01
6	1.318	2.46	0.18	0.24	0.06
7	2.773	1.89	0.12	0.16	0.04
8	3.503	3.71	0.08	0.08	0.00
9	0.560	0.69	0.06	0.04	0.02

[a] $\Delta R_F = R_{F(exp.)} - R_{F(calc.)}$.

The experimental and calculated R_F values for the components investigated are compared in Tables I–IV together with the gradient programmes used. The error, calculated as $\Delta R_F = R_{F(exp.)} - R_{F(calc.)}$, did not exceed 0.1 R_F units in 82.5% of the results. Only a few errors were in the range 0.1–0.2 and only one exceeded 0.2 R_F units, so that the agreement is satisfactory.

In the series of experiments an artificial mixture of glycosides available in this laboratory was used for the comparison of computer-calculated and experimentally determined R_F values (Table I). The mean error was only 0.03 R_F units. This is satisfactory considering the visual determination of the position of the spots under the UV lamp. Similar results were obtained for Cholesol extracts (Table IV) with eluents composed of heptane and diisopropyl ether (modifier); the mean error was 0.035 R_F units. The results for Seboren extracts with eluents composed of chloroform and ethyl acetate (modifier) were also satisfactory too; the mean error was 0.05 R_F units (Table III).

The results obtained for Seboren with diisopropyl ether as modifier were less satisfactory (Table II). The mean error was 0.11 R_F units. One reason was presumably the high capacity factor for some of the components. Another cause is that the Snyder–Soczewiński equation is more useful for narrow ranges of modifier concentrations; during the development program the modifier concentration was changed from the pure modifier to a concentration equal to 0.1 volume fraction.

CONCLUSIONS

The two-stage incremental gradient development mode of TLC resulted in good separations of mixtures of practical significance. The agreement between the predicted (on the basis of the Snyder–Soczewiński equation and eqn. 2 in this paper) and experimental R_F values was good to fair. The PTFE horizontal sandwich chambers of the DS type permit the use of incremental gradient development in a simple manner.

SYMBOLS

$c_{(i)}$ concentration of modifier in the ith step (volume fraction);
$c_{(mod)}$ concentration of modifier (volume fraction);
$z_{(i)}$ development distance in the ith step;
$R_{F(j)}$ final R_F value of solute j in gradient development;
$k_{0(j)}$ capacity factor of solute j for unit concentration of modifier (pure modifier) for normal-phase systems;
$m_{(j)}$ slope of log k' *vs.* log c plot for solute j;
$R_{F(i,j)}$ R_F value for solute j corresponding to ith concentration of modifier;

$k_{(i,j)}$ capacity factor of solute j for the ith step;
r correlation coefficient.

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