Journal of Chromatography, 518 (1990) 273–276
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 603

Note

Stepwise gradient in thin-layer chromatography of *Chelidonium* alkaloids

G. MATYSIK* and L. JUSIAK
Department of Inorganic and Analytical Chemistry, Medical Academy, ul. Staszica 6, 20081 Lublin (Poland)
(First received March 23rd, 1990; revised manuscript received May 30th, 1990)

The application of gradient elution in thin-layer chromatography (TLC) has been found to be very effective in the separation of complex mixtures, *e.g.*, plant extracts [1–9], whose components have a wide range of polarity. Various methods of gradient elution have been elaborated, for instance, by preadsorption of solvent vapours in chambers devised by Geiss and Schlitt (see ref. 1), Kaiser [10] and De Zeeuw [1,12] or by the use of eluent distributors as described by Niederwieser and Honegger [2,3], Soczewiński and co-workers [13–15] and more recently Dzido and Soczewiński [16,17].

In all gradient techniques, if there are large differences in the eluent strengths of the component solvents of the mobile phase [A (weaker) and B (stronger)], deformation of the gradient profile is observed, owing to much stronger adsorption of component B in the layer, which is especially significant at low concentrations of solvent B. This effect leads to accumulation of spots in the zone of strong changes in the composition of the mobile phase [13].

In order to eliminate this undesirable effect, a combined polyzonal–stepwise gradient programme was applied in a recent paper [18]. As in ordinary stepwise gradients, the eluent is composed of two solvents, A (weaker) and B (stronger), the content of solvent B being increased stepwise; however, one component (or both) is composed of several solvents (*e.g.*, B_1, B_2 and B_3) of differentiated eluent strength ε^0, so that $\varepsilon^0_{B1} < \varepsilon^0_{B2} < \varepsilon^0_{B3}$. Under these conditions, solvents B_1–B_3 undergo frontal chromatography, as in polyzonal elution, thus reducing the sudden changes in the eluent strength along the layer.

In this paper, an analogous ternary stepwise gradient programme was applied to the analysis of *Chelidonium* alkaloids in the waste alkaloid fractions after industrial isolation of two dominant alkaloids used in therapy, chelidonine and protopine [19]. The extracts contain alkaloidal derivatives of benzophenanthridine, protoberberine, protopine and aporphine; some of them have interesting pharmacological properties [20] such as anticancer and spasmolytic (chelidonine and protopine), antimicrobial (chelerithrine) and anti-inflammatory properties (sanguinarine).

0021-9673/90/$03.50

EXPERIMENTAL

The industrial waste alkaloid fraction was obtained from Herbapol (Wroclaw, Poland). Reference alkaloids were prepared in this Department according to several procedures [21–23]. A chloroform solution of the mixture of alkaloids was spotted without preliminary purification on precoated silica gel plates (Si 60, 5 × 10 cm; E. Merck, Darmstadt, F.R.G.). The separated spots were detected under UV light (366 nm) or with Dragendroff's reagent.

A horizontal sandwich chamber with a glass distributor was used [13,14], equipped with a spiral PTFE capillary serving as a reservoir of eluent fractions [15]. The gradient programmes applied are given in Table I. Portions of 0.2 ml of six eluent fractions were introduced into six small test-tubes and sucked consecutively into the PTFE capillary in the reverse order (6–1) by moving the plunger of the syringe [15] backwards; to avoid mixing, each fraction was separated by a small air bubble. After introducing fraction under the distributor of the chamber and starting the development, the consecutive fractions of increasing eluent strength were sucked under the distributor by capillary forces so that stepwise gradient elution was produced.

A simpler method [13,14] consists in direct introduction of the consecutive eluent fractions 1–6 under the distributor from a micropipette, after complete absorption of the previous fraction by the layer. The PTFE capillary is then not necessary.

RESULTS AND DISCUSSION

Fig. 1 presents the chromatograms of the waste alkaloid extract obtained for a binary, six-step gradient programme (Table I). The spots are accumulated in the lower part of the chromatogram and a distinct eluent demixing front is visible. The use of fractions containing higher concentrations of methanol did not improve the separation.

Fig. 2 is the chromatogram obtained with the first ternary stepwise gradient programme given in Table I and Fig. 3 that obtained with the second ternary gradient programme given in Table I. It can be seen that the use of ethyl acetate as a compo-

TABLE I

SIX-STEP GRADIENT ELUTION PROGRAMMES WITH BINARY AND TERNARY ELUENTS

Eluent	Solvent	Eluent fraction No.					
		1	2	3	4	5	6
Binary	A = toluene	99	98	95	93	90	88
	B = methanol	1	2	5	7	10	12
Ternary	A = toluene–ethyl acetate (1:1)	99	98	95	93	92	90
	B = isopropanol	1	2	5	7	8	10
Ternary	A = toluene–ethyl acetate (1:1)	100	98	97	95	93	90
	B = methanol	–	2	3	5	7	10

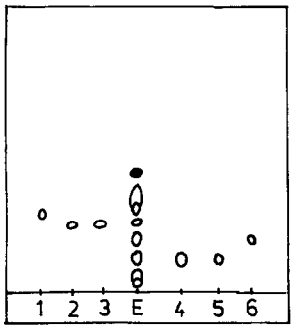
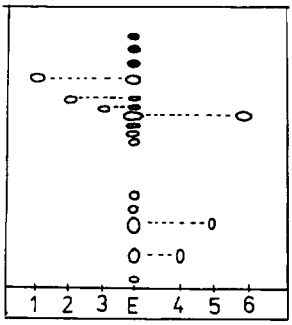


Fig. 1. Chromatogram of alkaloid extract (E) and reference solutes: (1) sanguinarine; (2) homochelidonine; (3) chelerithrine; (4) allocryptopine; (5) protopine; (6) chelidonine. Stepwise gradient according to Table I with binary eluent. White spots, visible under UV radiation and reacting with Dragendorff's reagent; black spots, visible only under UV radiation. Reference alkaloids, 4 μl of 0.1% (w/v) solution; extract, 8 μl of 0.5% solution.

nent of the mobile phase, owing to its moderate eluent strength, eliminated the effect of solvent demixing; the alkaloid spots are well shaped and compact, distributed along the whole chromatogram. Twelve spots reacting with Dragendorff's reagent are visible, including a large amount of chelidonine and trace amounts of three alkaloids (a, b and c) which could not be separated in isocratic systems and in systems reported in earlier papers [24,25]. These are presumably chelamine, chelamidine and coptisine [20]. The total number of separated spots visible under UV light is about 30. It is also noteworthy that two pairs of alkaloids are well separated in the system reported, *i.e.*, protopine–allocryptopine and chelerithrine–sanguinarine (pseudochelerithrine), with minor structural differences (dimethoxy or methylenedioxy groups). These pairs of alkaloids show small differences in R_F values in other systems [22–25].



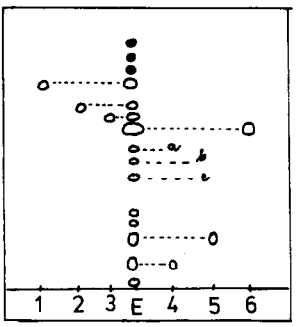


Fig. 2. As in Fig. 1, with gradient programme according to Table I using the first ternary eluent.

Fig. 3. As in Fig. 1, with gradient programme according to Table I using the second ternary eluent.

CONCLUSIONS

The separation efficiency in gradient TLC in the polyzonal–stepwise mode is much better, especially for complex samples containing solutes with a wide range of polarity, which requires a steep gradient with great differences in the polarities of component solvents A and B. Even the relatively simple ternary system is suitable also for the micropreparative TLC separation of alkaloids as reference compounds.

The method is suitable for direct TLC of waste alkaloid fractions without preliminary purification, which permits the detection of additional alkaloids present in trace amounts. It is also suitable for the analytical control of subsequent separation stages.

The concept of a polyzonal–stepwise gradient, where the component solvents A or B (or both) are mixtures of several solvents, can also be used in high-performance liquid chromatography for simple, two-compartment gradient generators.

REFERENCES

1 F. Geiss, *Fundamentals of Thin Layer Chromatography (Planar Chromatography)*, Hüthig, Heidelberg, 1987, p. 395.
2 A. Niederwieser and C. C. Honegger, *Adv. Chromatogr.*, 2 (1966) 123.
3 A. Niederwieser, *Chromatographia*, 2 (1969) 362.
4 G. Matysik and E. Soczewiński, *J. Chromatogr.*, 355 (1986) 363.
5 E. Soczewiński, G. Matysik and K. Glowniak, in R. E. Kaiser (Editor), *Instrumental High Performance Thin-Layer Chromatography, Proceedings of the 3rd international Symposium, Würzburg, April 1985*, Institut für Chromatographie, Bad Dürkheim, 1985, p. 413.
6 G. Matysik and E. Soczewiński, *Chromatographia*, 26 (1988) 178.
7 W. Cisowski, K. Glowniak, G. Matysik and E. Soczewiński, *Herba Pol.*, 33 (1987) 233.
8 T. Dzido, G. Matysik, E. Soczewiński, H. Wysokińska and U. Adamczyk, *Chromatographia*, 27 (1989) 24.
9 K. Glowniak, G. Matysik, M. Bieganowska and E. Soczewiński, *Chromatographia*, 22 (1986) 307.
10 R. E. Kaiser, in A. Zlatkis and R. E. Kaiser (Editors), *HPTLC—High Performance Thin-Layer Chromatography*, Elsevier, Amsterdam, 1977, p. 73.
11 *Desaga VP-Trennkammer nach De Zeeuw*, Druckschrift 171/71, Desaga, Heidelberg, 1971.
12 R. A. De Zeeuw, *Prog. Sep. Purif.*, 3 (1970) 1.
13 E. Soczewiński, in R. E. Kaiser (Editor), *Planar Chromatography*, Vol. 1, Hüthig, Heidelberg, 1986, p. 79.
14 G. Matysik and E. Soczewiński, *J. Chromatogr.*, 369 (1986) 19.
15 E. Soczewiński and G. Matysik, *J. Planar Chromatogr.*, 1 (1988) 354.
16 T. H. Dzido and E. Soczewiński, *J. Chromatogr.*, in press.
17 T. H. Dzido, *J. Planar Chromatogr.*, 3 (1990) 144.
18 E. Soczewiński and G. Matysik, *J. Planar Chromatogr.*, in press.
19 J. Jusiak, J. Kuczyński, E. Soczewiński, T. Gersz and S. Popiołek, *Pol. Pat.*, 261 306, 1985.
20 I. W. Southon and J. Buckingham (Editors), *Dictionary of Alkaloids*, Chapman and Hall, London, New York, 1989.
21 L. Jusiak, E. Soczewiński, J. Respondek, T. Żaba, M. Ciesielski and P. Sieradzki, *Pol. Pat.*, 129 822, 1981.
22 J. Jusiak, E. Soczewiński and M. Ciesielski, *Pol. Pat.*, 136 290, 1985.
23 L. Jusiak, A. Rompala, *Pol. Pat.*, 141 868, 1987.
24 L. Jusiak, *Acta Pol. Pharm.*, 39 (1982) 249.
25 B. Szabelska, L. Jusiak and G. Matysik, *Acta Pol. Pharm.*, 38 (1981) 329.