Journal of Natural Products
Vol. 49, No. 5, pp. 885-891, Sep-Oct 1986

PREPARATIVE ON-LINE OVERPRESSURE LAYER CHROMATOGRAPHY (OPLC): A NEW SEPARATION TECHNIQUE FOR NATURAL PRODUCTS

SZ. NYIREDY,[1] C.A.J. ERDELMEIER, K. DALLENBACH-TOELKE, K. NYIREDY-MIKITA, and O. STICHER*

School of Pharmacy, Swiss Federal Institute of Technology (ETH), 8092 Zurich, Switzerland

ABSTRACT.—Overpressure layer chromatography (oplc) is further developed for the on-line preparative isolation of natural compounds. Its use is demonstrated for the rapid isolation of one or two compounds in less than 1 h as well as for the isolation of more substances from plant extracts and/or prepurified fractions which require a few hours. Depending on the separation problem, sample sizes between 50 mg and 0.5 g may be applied to 20×20 cm silica plates. Principles of the method, practical considerations, and advantages of this techique over other available methodologies are discussed.

For many years, the most commonly used method in preparative planar chromatography has been traditional layer chromatography, requiring removal and subsequent elution of substance zones (1). Sequential thin layer chromatography (stlc) with locally and temporally variable solvent application (2,3) has also been useful for preparative purposes (4), even though it is an off-line method. One major advance in preparative planar chromatography was the development of centrifugally accelerated layer chromatography (clc), allowing on-line separations to be made. It has been shown, however, to be rather inefficient when used to solve complex separation problems (5).

Overpressure layer chromatography (oplc) is a forced-flow technique developed by Tyihák, *et al.* (6-8) in which the vapor phase is eliminated by completely covering the sorbent layer with an elastic membrane under external pressure. The separation is thereby carried out in a closed system with parameters that may be controlled. To date, there is information available on preparative oplc separations only in the off-line mode (9-11).

We now report the use of oplc on a 20- and 40-cm distance for preparative on-line separation of naturally occurring compounds.

METHODS

The chromatographic plate becomes a planar column by sealing the plate all around and by draining off the solvent. The eluent passes a detector system and is fractionated by an automatic collector. The flow scheme is depicted on Figure 1.

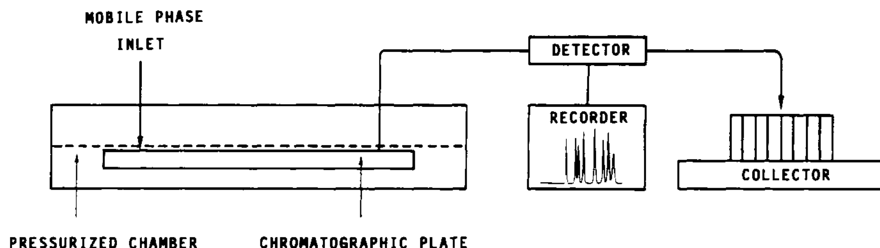

FIGURE 1. Flow scheme for preparative on-line oplc

[1]Visiting scientist, on leave from Semmelweis Medical University, Institute of Pharmacognosy, 1085 Budapest, Hungary.

Preparative on-line oplc requires special precoated plates, whose edges are obliquely scratched off and subsequently impregnated with a suitable polymer suspension (see 1 in Figure 2) in order to prevent solvent leakage at overpressure. Later, two thin channels are scratched out of the layer (see 2 and 3 in Figure 2). A second channel on the 20×20 cm plate is placed 18 cm from the inlet channel, permitting drainage of the eluent. The chromatographic plate thus prepared is shown in Figure 2 from above and in sideview.

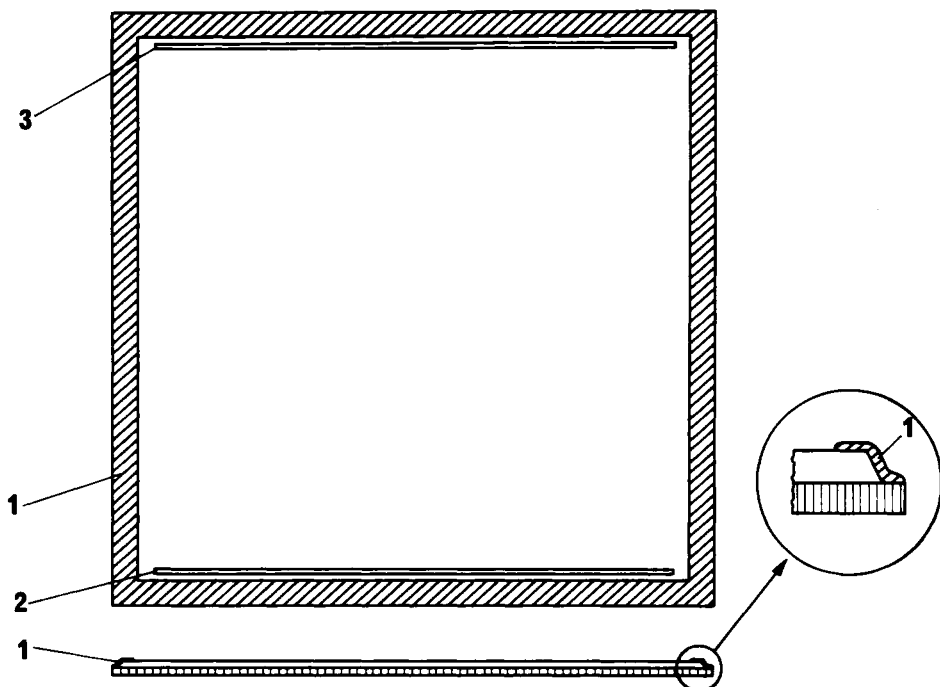

FIGURE 2. Preparation of the chromatographic plate
1=Polymer suspension
2=Inlet channel
3=Outlet channel

After sample application, the plate is placed into the chamber so that after closing, the solvent inlet is located on top of the inlet channel, and the solvent outlet on the outlet channel. Afterwards, the water cushion is set under pressure. Before initiating separation with a suitable mobile phase, the solvent inlet valve is closed, and the eluent pump is started so as to reach an appropriate solvent pressure. The separation is then initiated by opening the inlet valve, producing a quick solvent distribution into the channel resulting in a linear migration of the mobile phase. The eluent is forced through the silica layer and then collected in the outlet channel, passing through a detector and fractionated by a collector.

EXPERIMENTAL

All separations were carried out with a Chrompres-10 overpressure layer chromatograph (LABOR MIM, Budapest-Esztergom, Hungary). Samples were sprayed on with a Linomat III (CAMAG, Muttenz, Switzerland). For on-line detection and fractionation, an LKB Uvicord and a fraction collector were used (both from LKB, Bromma, Sweden). PSC precoated silica 60 $F_{254}S$ plates with a 2-mm layer thickness were obtained from MERCK (Darmstadt, W. Germany). Plate impregnation was performed with Impres polymer suspension from LABOR MIM. Reagent grade solvents were used for all separations. Frangula-emodin was isolated from an extract of *Rhamnus frangula* L. (Rhamnaceae). Extractum opii (Ph. Helv. VI) was used to demonstrate rapid isolation of papaverine and noscapine. The furocoumarins were separated

from an extract of *Heracleum sphondylium* L. (Umbelliferae) (12). The secoiridoids were isolated from a root bark extract of *Gentiana purpurea* L. (Gentianaceae).

RESULTS

RAPID ISOLATION OF NATURAL COMPOUNDS.—The first two oplc applications reported here were rapid isolations achieved within 1 h of relatively large amounts of major compounds. Such preparative separations are possible if the tlc mobile phase is optimized such that it brings the desired compounds into a medium Rf range, while other interfering substances mostly migrate in the solvent front or remain at the starting zone.

Isolation of frangula-emodin.—From a prepurified, hydrolyzed extract of *R. frangula*, 450 mg was taken for isolation of frangula-emodin. This separation is shown in Figure 3. The chrysophanic acid and physcion eluted quickly, virtually unseparated. Plant pigments and other interfering polar substances remained near the starting zone. Isolation was complete within 40 min, and 60 mg of pure frangula-emodin was obtained.

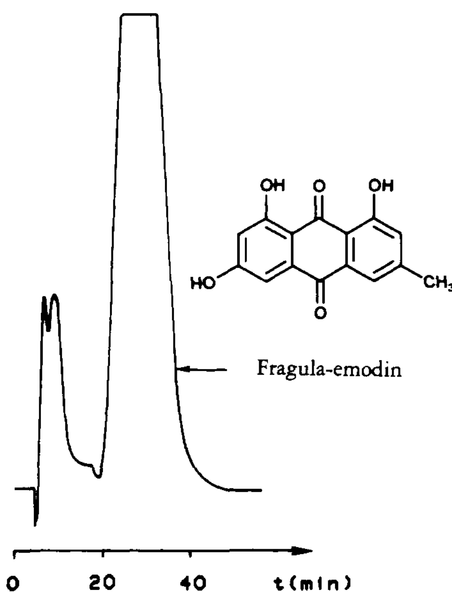

FIGURE 3. Rapid isolation of frangula-emodin

Conditions

Sorbent layer:	PSC precoated silica $60F_{254}$
Layer thickness:	2 mm
Separation distance:	17 cm
Mobile phase:	Et_2O-EtOAc-dioxane-hexane (11.9:7.8:6.9:73.4) (optimized with the "PRISMA"-model)
Flow rate:	5.7 ml/min
Cushion pressure:	12 bar
Detection:	UV 279 nm

Isolation of noscapine and papaverine.—Papaverine and noscapine were isolated from Extractum opii siccum (Ph. Hel. VI). From a 450-mg extract, 13 mg papaverine and 32 mg noscapine were isolated within 40 min at a separation distance of 17 cm and using a layer thickness of 2 mm (Figure 4).

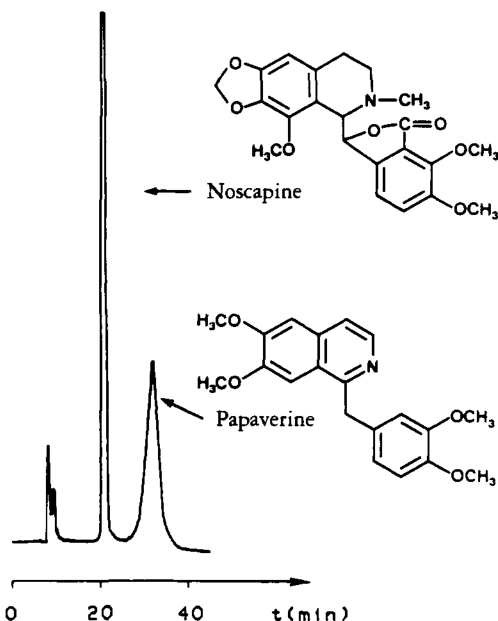

FIGURE 4. Rapid isolation of noscapine and papaverine

Conditions

Sorbent layer:	PSC precoated silica $60F_{254}$
Layer thickness:	2 mm
Separation distance:	17 cm
Mobile phase:	hexane-EtOAc-methoxyethanol (70:20:10)
Flow rate:	5.6 ml/min
Cushion pressure	10 bar
Detection:	UV 254 nm

The mobile phase contained methoxyethanol, which is appropriate for the separation of alkaloids (13), and was optimized for the separation of these two compounds by using the "PRISMA"-optimization model (14). A large proportion of the other main components (e.g., morphine) migrated very slowly and were not evident in the chromatogram.

PREPARATIVE SEPARATION OF NATURAL COMPOUNDS.—The next two on-line oplc separations are examples of the isolation of three or more compounds of similar chemical structure. For the preparative separation of natural compounds, between 50 and 200 mg of sample were applied with separation times of between 2 and 4 h.

Separation of furocoumarin isomers.—A $CHCl_3$ extract of *H. sphondylium* roots was prepurified, giving a concentrated fraction that contained five methoxylated furocoumarins. From a 50-mg extract, 4.8 mg isobergapten, 15.5 mg pimpinellin, 2.8 mg bergapten, 7.2 mg isopimpinellin, and 3.1 mg sphondin were obtained. As may be seen from Figure 5, the separation was satisfactory with only a few mixed fractions, but it required about 3 h.

Separations of secoiridoid glycosides.—The four main bitter constituents of *G. purpurea* were isolated on a 20×40 cm precoated silica plate with a layer thickness of 2 mm and a separation distance of 36 cm. A mobile phase containing equal parts of MeOH, $CHCl_3$, and THF was used, and the solvent strength (S_T) was adjusted with hexane (14). The three main compounds amaropanin, amarogentin, and gentiopicroside and the minor

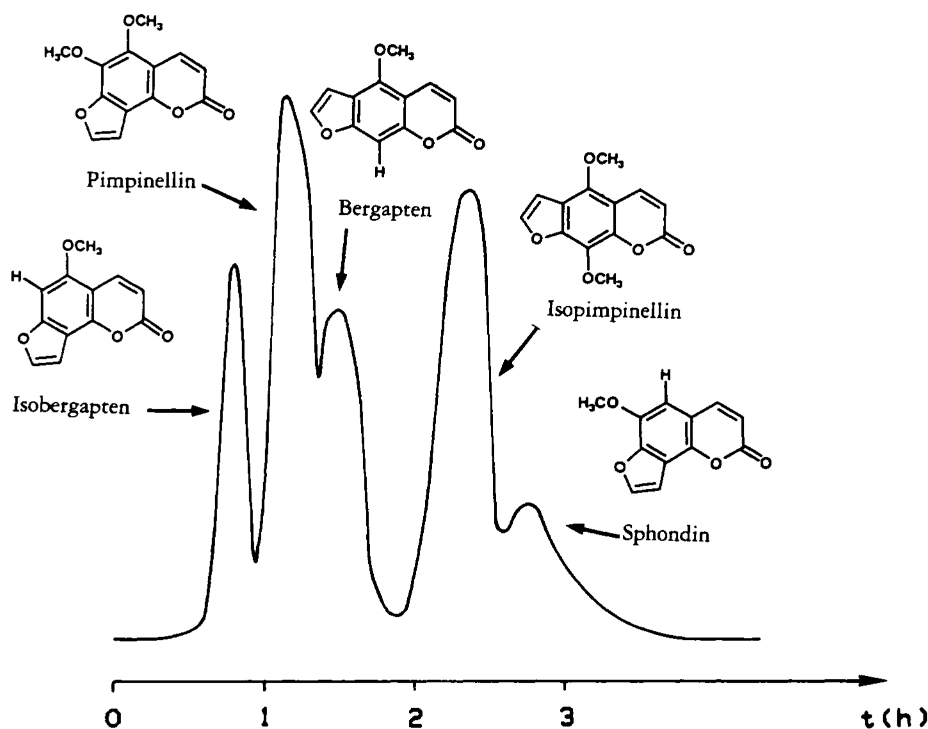

FIGURE 5. Separation of furocoumarin isomers

Conditions

Sorbent layer:	PSC precoated silica $60F_{254}S$
Layer thickness:	2 mm
Separation distance:	17 cm
Mobile phase:	CH_2Cl_2-THF-$CHCl_3$-hexane (8.1:6.3:6.1:79.5) (optimized with the "PRISMA"-model)
Flow rate:	5.7 ml/min
Cushion pressure	12 bar
Detection:	UV 313 nm

component amaroswerin were isolated with a three-step gradient (Figure 6). After 130 min, the solvent strength of mobile phase was increased from $S_{T_1}=1.86$ to $S_{T_2}=2.58$. Following the elution of amaroswerin, the solvent strength was again increased to $S_{T_3}=3.3$. From a 300-mg extract, 4.5 mg amaropanin, 57.8 mg amarogentin, 2.3 mg amaroswerin, and 74.2 mg gentiopicroside were obtained.

If only gentiopicroside is to be isolated on a 20×20 cm plate, the solvent strength of the mobile phase then has to be 2.7. In this case, 74.9 mg of gentiopicroside was isolated from 300 mg extract within 1 h.

PRACTICAL CONSIDERATIONS

The most important consideration in working with preparative on-line oplc is the preparation of a selected chromatographic plate. The impregnation must be carried out in such a way that the plate is compactly sealed all around, thereby obtaining a "planar column." This is achieved by scraping off the plate edges to a width of 3 mm and then polishing the adsorbent to obtain an oblique profile at the edges (see Figure 2). The uncoated glass and layer edges of the plate are then impregnated with the polymer suspension to a width of 7-8 mm and allowed to dry at room temperature for several hours.

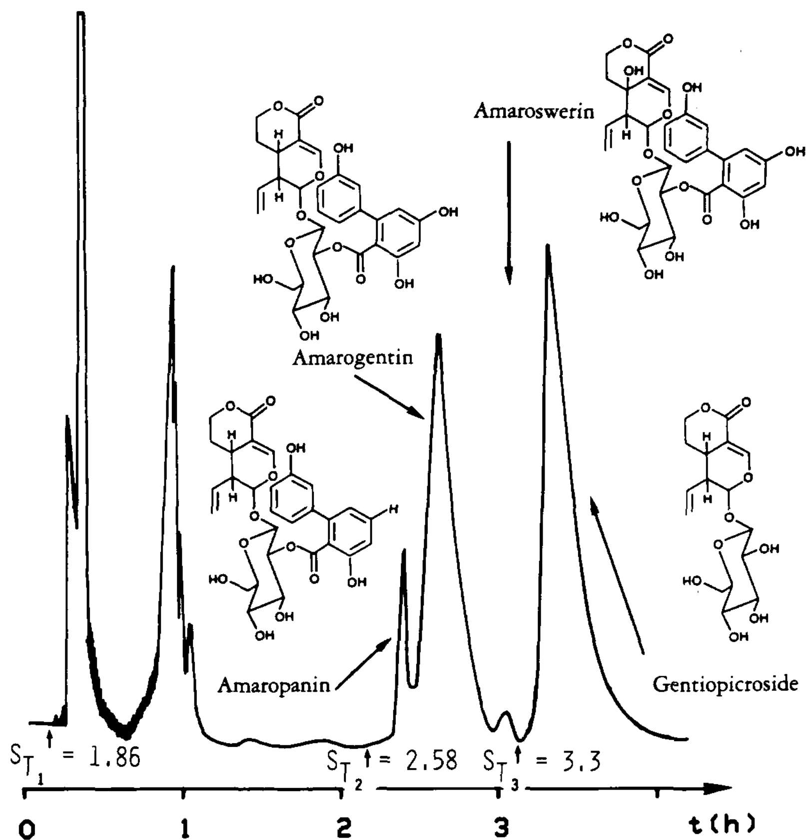

FIGURE 6. Separation of secoiridoid glycosides

Conditions

Sorbent layer:	PSC precoated silica $60F_{254}$
Layer thickness:	2 mm
Separation distance:	35 cm
Mobile phase:	MeOH-$CHCl_3$-THF-hexane
	S_{T_1}=1.86 (12.2:15.1:15.5:57.2)
	S_{T_2}=2.58 (16.9:20.9:21.5:40.7)
	S_{T_3}=3.3 (21.6:26.8:27.5:24.1)
	(optimized with the "PRISMA"-model)
Flow rate:	5.7 ml/min
Cushion pressure	10 bar
Detection:	UV 254 nm

This impregnation guarantees a tight fitting of the plastic membrane to the edges when the solvent is pressed through the plate. Both channels, solvent inlet, and drainage from the layer should have a width of about 0.5 mm.

Before starting a separation, one must be certain that the solvent inlet exactly coincides with the inlet channel; otherwise, the mobile phase is not uniformly distributed. This also happens when the initial solvent speed is too low.

A teflon sheet is inserted between the water cushion and the chromatographic plate to protect the cushion foil against aggressive solvents. After each run, the protective sheet is removed from the cushion, placed between filter papers, and thermally treated for a few minutes to remove traces of solvents. To avoid problems with the uv-detector, only degassed solvents should be used, and, if possible, a prerun with hexane should be made in order to eliminate any air and/or gas within the adsorbent. Since plates for pre-

parative purposes are coated with rather coarse adsorbent material with average particle sizes of about 25 μm and a size distribution of 5-40 μm, the transfer of the mobile phase from the analytical to the preparative oplc assay may be improved by reducing the solvent strength of the mobile phase (11,15).

DISCUSSION

This paper describes a new on-line preparative planar chromatographic method that is a valuable addition to methods used for the isolation of naturally occurring compounds. The eluted compounds are drained from the plate, thereby allowing for the connection of a flow detector, for recording of chromatograms, and for collection of separated compounds with a fraction collector. The separation efficiency of this method may be further improved by the application of mobile-phase, flow-rate, and/or temperature gradients (11). Samples sizes may range between 50 mg and 0.5 g, depending on the separation problem. Rapid isolation of one or two compounds can be completed within 1 h, while more difficult separations (e.g., separation of isomers) can be obtained within a few hours.

Oplc may be compared with other planar chromatographic techniques such as preparative layer chromatography and clc. One of the major advantages of on-line oplc is that all of the separated compounds migrate over the entire length of the adsorbent (20 or 40 cm), and that the separation distance is two to five times longer than in clc; therefore, the resolution is significantly greater than that obtained by preparative layer chromatography or clc, especially in the lower Rf range. The mobile phase from the tlc-preassays may also be more easily transferred to oplc than to clc since it is a closed system with negligible solvent loss due to evaporation (16). Both resolution and reproducibility are better than in clc and preparative layer chromatography, and diffusion is reduced due to the linear velocity of the mobile phase.

ACKNOWLEDGMENTS

A research grant from the Swiss National Science Foundation is gratefully acknowledged. The authors also thank LABOR MIM, Budapest-Esztergom, Hungary, for loan of the Chrompres-10 oplc instrument.

LITERATURE CITED

1. H.R. Felton, in: "Advances of Thin Layer Chromatography." Ed. by J.C. Touchstone, John Wiley Sons, New York, 1982, pp. 13-20.
2. P. Buncak, *GIT Suppl. Chromatographie,* 3 (1982).
3. P. Buncak, *Fresenius Z. Anal. Chem.*, **318**, 289 (1984).
4. P. Buncak, *Fresenius Z. Anal. Chem.*, **318**, 291 (1984).
5. C.A.J. Erdelmeier, Sz. Nyiredy, and O. Sticher, *HRC & CC,* **8**, 132 (1985).
6. E. Tyihák, E. Mincsovics, and H. Kalász, *J. Chromatogr.*, **174**, 75 (1979).
7. E. Mincsovics, E. Tyihák, and H. Kalász, *J. Chromatogr.*, **191**, 293 (1980).
8. Sz. Nyiredy, C.A.J. Erdelmeier, and O. Sticher, in: "Proceedings of the International Symposium on TLC with Special Emphasis on Overpressured Layer Chromatography (OPLC)." Ed. by E. Tyihák. Szeged, Hungary, September 10-12, 1984, LABOR MIM, Budapest, 1986, pp. 222-231.
9. T.J. Székely and E. Mincsovics, "Advances in Liquid Chromatography," Szeged, Hungary, 1984, Abstr. p. 84.
10. P. Oroszlán, G. Verzár-Petri, E. Mincsovics, T. Székely, and A. Vastagh, "Advances in Liquid Chromatography," Szeged, Hungary, 1984, Abstr. p. 65.
11. Sz. Nyiredy, C.A.J. Erdelmeier, and O. Sticher, Instrumental Preparative Planar Chromatography, in: "Planar Chromatography, Vol. 1." Ed. by R.E. Kaiser, Heidelberg, Hüthig Verlag, 1986, pp. 119-164.
12. C.A.J. Erdelmeier, Ph.D. thesis No. 7294, ETH Zürich (1983).
13. Sz. Nyiredy, C.A.J. Erdelmeier, and O. Sticher, *Fresenius Z. Anal. Chem.*, **321**, 556 (1985).
14. Sz. Nyiredy, C.A.J. Erdelmeier, B. Meier, and O. Sticher, *Planta Med.*, No. 3, 241 (1985).
15. Sz. Nyiredy, C.A.J. Erdelmeier, B. Meier, O. Sticher, and K. Mikita, International Research Congress on Natural Products, Chapel Hill, NC, 1985, Abstr. No. 124.
16. Sz. Nyiredy, S.Y. Mészáros, K. Nyiredy-Mikita, K. Dallenbach-Tölke, and O. Sticher, *GIT Suppl. Chromatographie,* No. 3, 51 (1986).

Received 26 March 1986