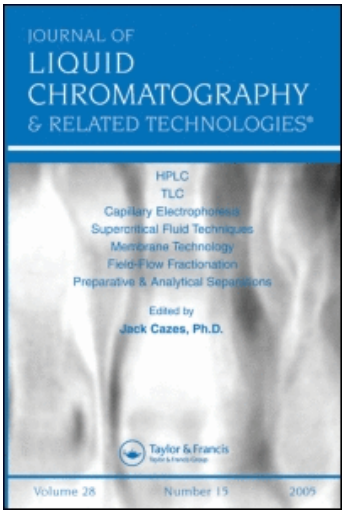

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Kalász, Huba , Hosztafi, Sándor , Csermely, Tamás , Götz, Henrik and Szabó, Marián G.(1996) 'Displacement Thin Layer Chromatography of Morphine and Its Semi-Synthetic Derivatives', Journal of Liquid Chromatography & Related Technologies, 19: 1, 23 — 35
To link to this Article: DOI: 10.1080/10826079608006287
URL: http://dx.doi.org/10.1080/10826079608006287

J. LIQ. CHROM. & REL. TECHNOL., 19(1), 23–35 (1996)

DISPLACEMENT THIN LAYER CHROMATOGRAPHY OF MORPHINE AND ITS SEMI-SYNTHETIC DERIVATIVES

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ABSTRACT

Morphine and its sixteen semisynthetic derivatives of pharmacological interest were subjected to planar chromatography. Adequate stationary and mobile phases for both elution and displacement chromatography were found. All these components can be separated by elution mode of development. At the same time, several morphine derivatives can be the member of displacement train. Experiments and results using either straight-phase or reverse phase chromatography with both elution and displacement types of developments are detailed.

INTRODUCTION

Displacement chromatography is based on the well known phenomenon that the more strongly retarded compounds can displace the less strongly retarded ones, as it has been known for a long time[1]. In 1943, Tiselius classified chromatographic separation according to the mode of development, such as elution, frontal and displacement chromatography[2], which was widely used in the forties and early fifties for separation of various compounds, such as proteins, peptides, amino acids, etc.[3-5].

The overwhelming majority of these separations was done using column arrangement of the stationary phase, and this column displacement chromatography was adopted to the high performance liquid chromatographic conditions by Horváth et al. in 1981[6] when they separated numerous types of organic compounds using high performance displacement chromatography. At the same time, their experiments and publications have started an essential boom of displacement separations for preparative purposes. In 1982, displacement chromatography on planar arrangement of the stationary phase was used to scout the optimum conditions for high performance displacement separations[7], and this effort was continued through the '80s[8-12].

Spacer displacement thin layer chromatography (SD-TLC) employs spots or bands of component(s) which are inserted between the two displaced components to improve their separations. Thereby resolution of displacement thin layer chromatography of some drugs and metabolites was increased, and the detection of the tiny bands of the displaced components was made easier without the use of the spacers.

Detailed accounts on the displacement chromatography of ecdysteroids (insect moulting hormones that can be found in insects and also in plants) were published to demonstrate the separating power of planar displacement chromatography, as well as the application of forced flow development at planar displacement chromatography.[13-15]

Our recent research[16] has been focused on the chromatographic behaviour of morphine and their derivatives with pharmacological interest[17]. To determine hydrophobicity of morphine derivatives, a series of reverse phase and straight phase TLC plates were checked, some of them were originated from commercial sources, others were home-made[16]. Our investigations were expanded to find conditions for displacement chromatography.

Table 1

The Structures of Compounds that Were Investigated

		------ Substituents, such as ------				
Compound		**R_1 N**	**7-8**	**R_2 6**	**R_3 3**	**R_4 14**
Morphine	(B)	$-CH_3$	=	-OH	-OH	-H
Azidomorphine	(C)	$-CH_3$	DH	$-NH_3$	-OH	-H
ECAM	(D)	$-CH_2$-cPr	DH	$-NH_3$	-O-Et	-H
14-Hydroxy-dihydro-morphine	(E)	-Met	DH	-OH-	-OH	-OH
Dihydromorphine	(F)	-Met	DH	-OH-	-OH	-H
Norazido-ethyl-morphine	(G)	-H	DH	$-N_3$	-O-Et	-H
CAM	(H)	$-CH_2$-cPr	DH	$-N_3$	-OH	-H
14-Hydroxy-azido-codeine	(I)	-Met	DH	$-N_3$	-O-Met	-OH
14-Hydroxy-azido-morphine	(J)	-Met	DH	$-N_3$	-OH	-OH
Ethylmorphine	(K)	-Met	=	-OH	-O-Et	-H
Norazidomorphine	(L)	-H	DH	$-N_3$	-OH	-H
6-Amino-dihydro-morphine	(M)	-Met	DH	$-NH_2$	-OH	-H
Normorphine	(N)	-H	=	-OH	-OH	-H
Nalorphine	(O)	-allyl	=	-OH	-OH	-H
Codeine	(P)	-Met	=	-OH	-O-Met	-H
Azidocodeine	(Q)	-Met	DH	$-N_3$	-O-Met	-H
14 Hydroxy-dihydro-codeine	(R)	-Met	-DH	-OH	-O-Met	-OH

Abbr.: DH: dihydro -cPr: -cyclopropyl
-Met: -methyl -Et: -ethyl
=: double bond

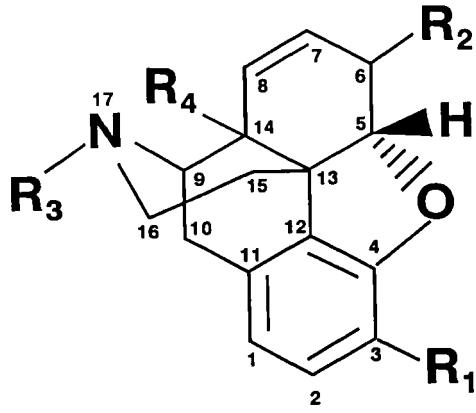

Figure 1. The structure of morphine and its derivatives. The substituents are listed in Table 1.

EXPERIMENTAL

Stationary phases were purchased from E. Merck (Darmstadt, Germany). TLC plates silica gel 60 F_{254} pre-coated on glass, 20x20 cm, layer thickness 0.25 mm (Art. No. 5715), and TLC plates RP-18 $F_{254}S$, pre-coated on glass, 20x20 cm, layer thickness 0.25 mm (Art. No. 15389) were used.

Mobile phases for displacement development contained triethanolamine (alt., Reanal, Budapest, Hungary) or tetrabutylammonium chloride (pract., Kat. No. 86870, Fluka, Buchs, Switzerland) for straight phase or reverse phase displacement chromatography, respectively. Solvents were purchased from commercial sources in the highest available quality.

Morphine and its semisynthetic derivatives were kind gifts of Alkaloida Pharmaceutical Works (Tiszavasvári, Hungary). The structures of morphine derivatives used for separations are given in Fig. 1 and Table 1.

All TLC developments were performed in glass chambers (Desaga, Heidelberg, Germany) at ambient temperature. After developments, the TLC plates were dried and spots were visually detected at daylight as well as under UV light of 254 nm (using UV lamp of Desaga).

RESULTS

Fig. 2 gives the separation of morphine derivatives using elution type development on silica stationary (straight) phase and with several different running systems.

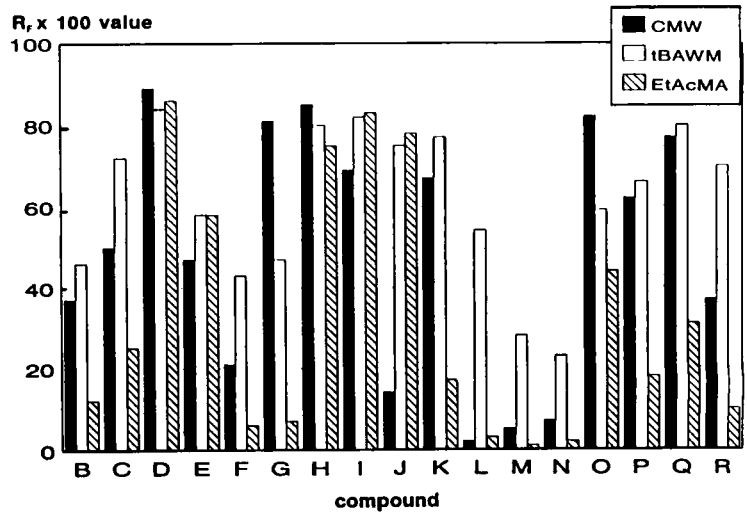

Figure 2. RF values that characterize thin layer chromatography of morphine and its derivatives on silica stationary phase using chloroform-methanol-water (7:5:1, v/v), t-butanol-ammonia-water-methanol (20:1:4:2, v/v) and ethyl acetate-methanol-ammonia (18:2:1, v/v) mobile phases. B. C, D, ..., R refer the compounds given in Table 1.

Fig. 3 gives TLC of morphine derivatives using n-butanol-0.2 M hydrochloric acid-water-methanol (20:1:4:2, v/v); some spots were displaced, others were eluted by the mobile phase. The eluted spots were both under and over the displacer front.

Figs. 4-6 give displacement chromatography of morphine derivatives on silica using chloroform, dichloromethane, dichloroethane carrier and triethanolamine displacer.

Fig. 7 gives displacement development on reverse phase stationary phase. Not all the morphine derivatives were displaced by the displacer front,

as several components remained behind the displacer front.

DISCUSSION

Thin-layer chromatography of morphine and its derivatives has been widely used to differentiate these compounds (for reviews, see[19-20]). Running systems with three or more solvents can be preferentially used, and the proper choice of the mobile phase can be made in knowledge of the main component(s) to be best separated from the others. Moreover, the use of several analyses in various running solvents can improve both the qualitative and quantitative evaluation of the results. The R_F values indicated in Fig. 2. show that practically any morphine derivatives can be well separated. and the identification of the morphine derivatives can be adequately done using all three different mobile phases given here.

Another possibility is to use an adequate ratio of the mixtures of n-butanol-acetic acid-water-methanol and n-butanol-ammonia-water-methanol[21]. Thereby changing the ratio of the running solvents containing acidic (acetic acid) and basic (ammonia) constituents, the different compounds show diverging dependence, which also serves for the optimization of separations. It is important to avoid the use of nonvolatile components, such as sodium- or phosphate ion containing buffers, because of their very limited migration with the mobile phase through the silica stationary phase. In this case, the various morphine derivatives migrate with very different R_F values, and the displacer can be easily removed from the zones of components which are eluted by the displacer (that is that components migrate slower then the displacer front, thereby their zones are contaminated with the displacer).

Fig. 3 shows TLC of morphine derivatives on silica using n-butanol-0.2M hydrochloric acid-water-methanol (20:1:4:2, v/v) mobile phase. While some morphine derivatives were eluted with the mobile phases, several components become part of the displacement train, that is they were displaced by the displacer train. This system has two basic advantages. First of all, the eluted components were either behind or ahead of the displacer front. Thereby, the whole plate was well utilized by the components showing various mobility. The second advantage is related to the composition of the displacing system, i.e. all components of the mobile phase can be easily removed if any one of the separated components is to be prepared. At the same time, this system

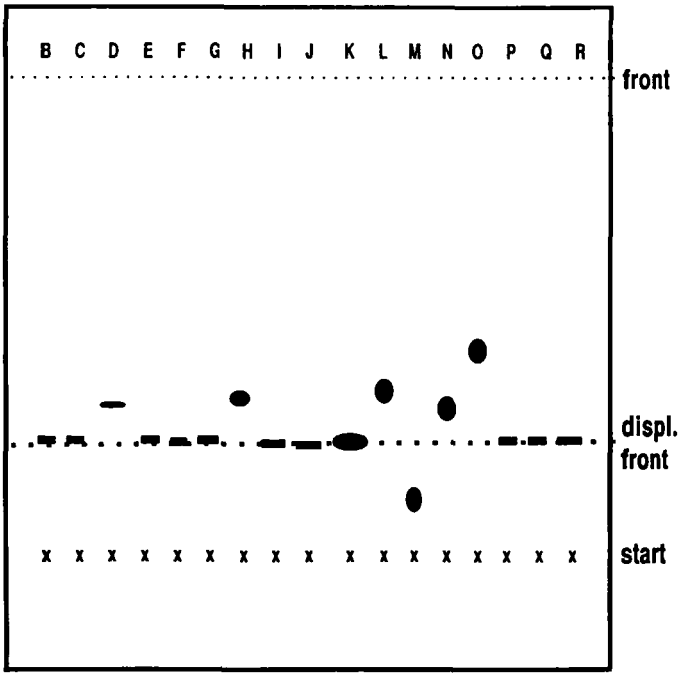

Figure 3. Thin layer chromatography of morphine and its derivatives on silica stationary phase using n-butanol-hydrochloric acid-water-methanol (20:1:4:2, v/v) mobile phase.

(n.butanol-0.2M hydrochloric acid-water-methanol (20:1:4:2, v/v)) is rather sensitive to the changes of temperature, which should be kept at 22 $\pm$ 2 °C, for reproducible results.

Figs. 4, 5, and 6 give displacement chromatogram of the morphine derivatives (see Table 1.) on silica stationary phase using chloroform, dichloromethane and dichloroethane carriers, respectively. Neither morphine, nor any of the morphine derivatives showed any migration when the plain carrier was used as mobile phase (not shown), however, all of these components migrated when the adequate amount of triethanolamine displacer was added to the mobile phase. Neither carrier was selective with any of the morphine derivatives, as norazidomorphine, normorphine and 6-amino-dihydromorphine were not displaced by the triethanolamine using either chloroform, or

dichloromethane or dichloroethane, while all other compounds were displaced by triethanolamine.

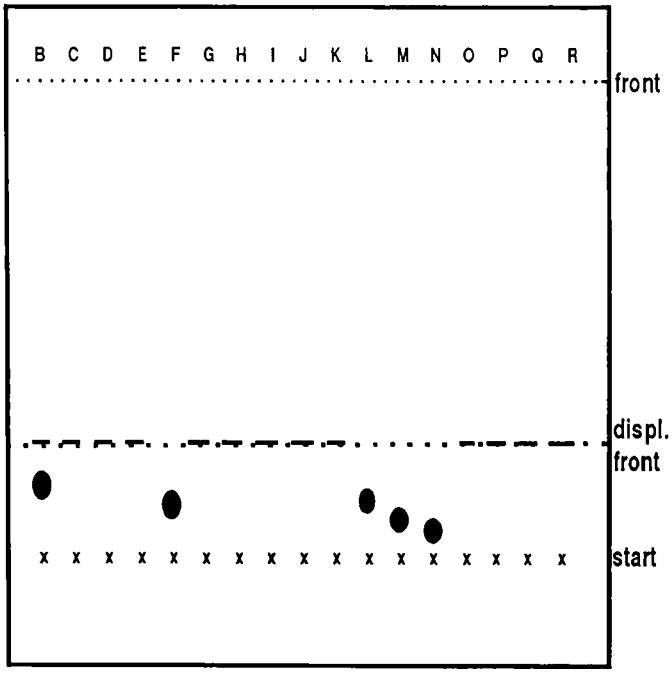

Figure 4. Thin layer displacement chromatography of morphine derivatives on silica using chloroform carrier and triethanolamine displacer.

The ratio of displacer to carrier was 5:95, 3:97 and 10:90 (v/v), in the experiments presented in Figs. 4, 5 and 6, respectively. However, displacers with lower (1:99 v/v) and higher (20:80) ratio could also generate fully developed displacer train after a relatively short development of the displacer front (not shown).

Planar displacement chromatography has hitherto been restricted to the use of straight stationary phases (silica[9-14] and alumina layers[12]). Fig. 7 presents displacement thin layer chromatography of some morphine derivatives on reverse phase stationary phase, that is on TLC plates RP-18 $F_{254}S$ (E. Merck). Our efforts to generate displaced bands using non-polar stationary

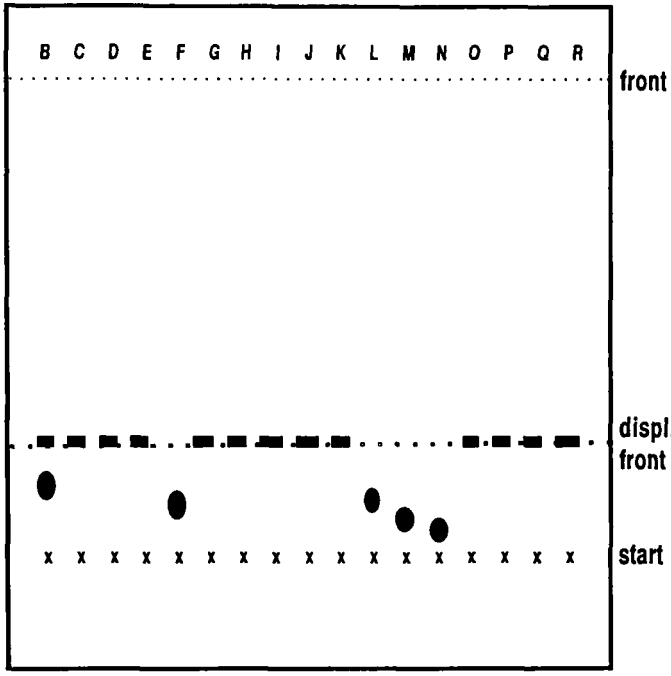

Figure 5. Thin layer displacement chromatography of morphine derivatives on silica using dichloromethane carrier and triethanolamine displacer.

phases have earlier been unsuccessful, although several other reverse phase plates were tried, such as other commercially available reverse phase TLC plates as well as home made, paraffin-coated silica plates. Although the paraffin impregnated silica plates were successfully used at the determination of hydrophobicity of morphine derivatives, displacement chromatography on these plates has never given adequate results. The basic problem was that morphine and its derivatives did not migrate, but remained in the place of their origin.

We suppose that non-migration of these spots is related to the limited wettability of these plates with aqueous-organic mobile phases, and the special surface characteristics of the non-polar stationary phases on the TLC plates RP-18 $F_{254}S$ (Merck) somehow circumvented the problem of too strong adsorption

of morphine derivatives to the surface of the stationary phase, like with the wettability of the RP-18 stationary phase with aqueous eluent (where the percentage of water is even higher than 80%).

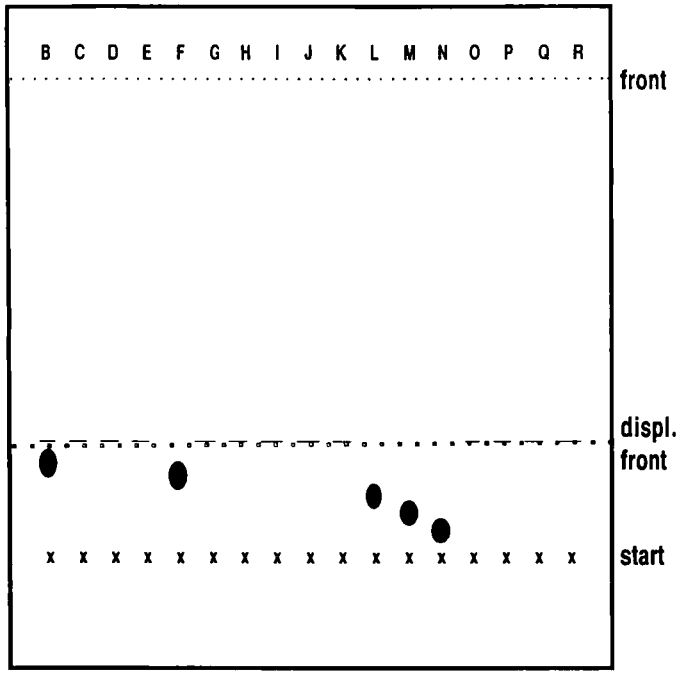

Figure 6. Thin layer displacement chromatography of morphine derivatives on silica using dichloroethane carrier and triethanolamine displacer.

The use of reverse phase stationary phases for displacement chromatography has recently found new important applications for the separation of macromolecules, such as peptides, proteins and nucleic acids on a preparative scale[24-29]. Preparative (e.g. industrial) scale separations require optimization of the conditions of displacement chromatography which can be conveniently done using planar stationary phases. Recently, reverse phase planar displacement chromatography has been performed with forced-flow of the mobile phase[30]. Thereby the development of the displacement train is faster, more effective and more reproducible, than propagation of the mobile phase by capillary forces.

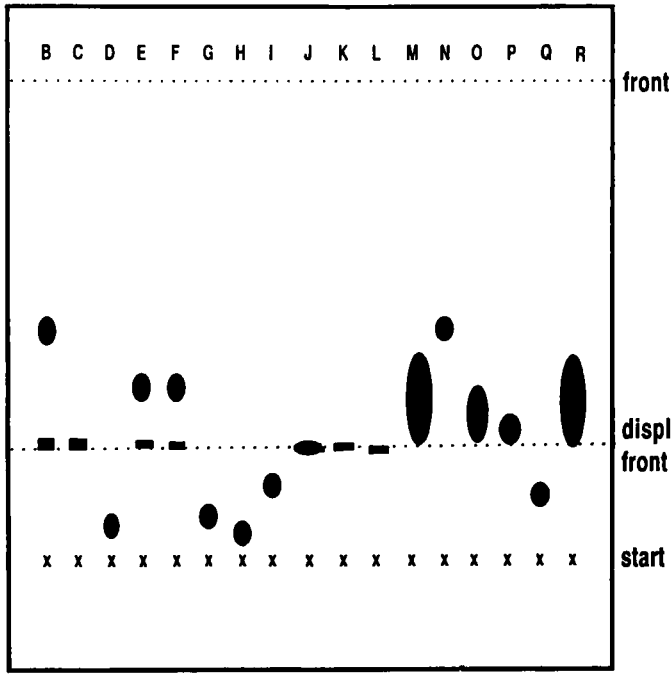

Figure 7. Reverse phase thin layer displacement chromatography of morphine derivatives on RP-18 F_{254} S TLC plates using 10% (w/v) tetrabuthylammonium chloride displacer in acetonitrile-water (30:70 v/v) carrier.

ACKNOWLEDGMENTS

This work was supported by grant No. 14445 of the Hungarian Academy of Sciences for HK. We thank Prof. Dr. J. M. Varga for his valuable advice.

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Received April 22, 1995
Accepted June 18, 1995
Manuscript 3862