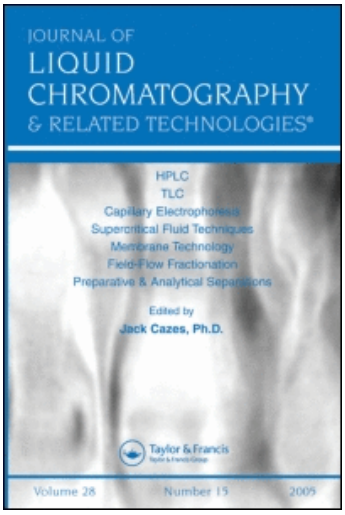

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Retention Behaviour of Triphenylethylene Derivatives in Reverse Phase Liquid Chromatography

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RETENTION BEHAVIOUR OF TRIPHENYLETHYLENE DERIVATIVES IN REVERSE PHASE LIQUID CHROMATOGRAPHY

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ABSTRACT

A series of triphenylethylene derivatives was studied with different reverse phase liquid chromatographic columns. Octyl and octadecyl silica stationary phases were compared, as well as, one type of polymeric reverse phase. Because these triphenylethylene derivatives appear highly lipophilic, the polymeric column (PRP-1) did not produce satisfactory peaks. Also, the basic nature of these molecules requires such column properties that were most satisfactorily met by well end capped octyl silane phase columns, like Kromasil C_8 or deactivated Supelco LC-8-DB. These columns will be suitable for evaluating lipophilicity data of triphenylethylene derivatives, in order to use them in quantitative structure-activity relationship studies.

INTRODUCTION

Triphenylethylene compounds represent a new source of important drugs for cancer chemotherapy. Tamoxifen, (Z)-4-2-[4-(1,2-diphenylbut-1-enyl)phenoxy]N,N-dimethylethylamine, and toremifene, (Z)-2-[4-(4-chloro-1,2-diphenylbut-1enyl)phenoxy]ethyidimethylamine, are well

known anticancer agents used in the case of breast tumours.[1-2] From the analytical point of view, there is not much data available concerning triphenylethylene derivatives.

For tamoxifen, some analytical methods have been published, including high performance liquid chromatography (HPLC),[3-4] GC-MS[5] and LC-MS.[6] Only a few analytical methods have been described for toremifene: HPLC with fluorescence,[7] ultraviolet light[8] and mass spectrometric detection.[9]

Tamoxifen and toremifene are highly lipophilic compounds, logarithms of apparent partition coefficients being 6.64 and 6.35, respectively.[10] Moreover, they are weakly basic organic molecules, e.g. pKa-value is 7.82 for tamoxifen.[11] For these reasons, the reverse phase liquid chromatography of these molecules and their analoques is not simple.[12-13]

In this investigation, reverse phase liquid chromatographic properties of twelve triphenylethylene derivatives and related compounds have been studied. Most of them are new ones, thus analytical data concerning these compounds has not been published earlier. This study is a preliminary outline about the RPLC properties of these molecules, the main aim being to investigate the lipophilicity of them later on.

Lipophilicity data shall be pronounced by log k values and, therefore, studied by RPLC, because conventional shake-flask method is not a suitable approach for these highly lipophilic compounds. Further, log k values are used in quantitative structure-activity relationships (QSAR) studies performed by comparative molecular field analysis (CoMFA) methods,[14] in order to develop new drug molecules.

EXPERIMENTAL

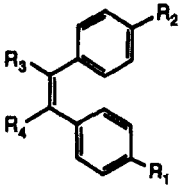
Materials and Chemicals

The triphenylethylene derivatives (Table 1) were obtained from Farmos Laboratories, Orion-Farmos Ltd (Turku, Finland). Standard compounds for lipophilicity correlation studies were of analytical grade (Table 2).

HPLC grade acetonitrile and methanol were purchased from Labscan Ltd (Dublin, Ireland). Ammonium acetate (Merck, Darmstadt, Germany) and triethylamine (Aldrich, Steinheim, Germany) were of analytical grade.

Table 1

Structures of Triphenylethylene Derivatives



	R_1	R_2	R_3	R_4
1. Toremifene E-isomer cirate	H	$OCH_2CH_2N(CH_3)_2$	CH_2CH_2Cl	H
2. Deaminocarboxyltorefene	OCH_2COOH	H	CH_2CH_2Cl	C_6H_5
3. Deaminohydroxytoremifene	OCH_2CH_2OH	H	CH_2CH_2Cl	C_6H_5
4. Toremifene citrate b. 16213P	$OCH_2CH_2N(CH_3)_2$	H	CH_2CH_2Br	C_6H_5
5. Fc-1158a citrate	$OCH_2CH_2N(CH_3)_2$	H	CH_2CH_2Br	C_6H_5
6. Demethyltoremifene citrate	$OCH_2CH_2NHCH_3$	H	CH_2CH_2Cl	C_6H_5
7. Fc-1530	H	OH	CH_2CH_3	cyclopentyl
8. Didemethyltoremifene hydrochloride	$OCH_2CH_2NH_2$	H	CH_2CH_2Cl	C_6H_5
9. 4-hydroxytoremifene	$OCH_2CH_2N(CH_3)_2$	H	CH_2CH_2Cl	C_6H_5OH
10. Fc-1530 b citrate	$OCH_2CH_2N(CH_3)_2$	H	CH_2CH_3	cyclopentyl
11. Fc-1159a citrate	$OCH_2CH_2N(CH_3)_2$	H	CH_2CH_3I	C_6H_5
12. Toremifene citrate	$OCH_2CH_2N(CH_3)_2$	H	CH_2CH_2Cl	C_6H_5

Table 2

Chemicals Used as References for log K-log P Correlation Studies

Compound	Source	log P*
Caffeine	Merck, Darmstadt, F.R.G.	-0.07
Diphenydramine	Sigma, St. Louis, MO	3.27
Isoniazide	Orion Corp., Espoo, Finland	-1.14
Quinine	Sigma	1.73
Salicylamide	University, Pharmacy, Finland	0.89
Sulfanilamide	Tamro Ltd, Helsinki, Finland	-0.72
Thioridazine	Star Ltd, Tampere, Finland	5.79
Toremifene	Orion-Farmos Corp., Finland	6.35
Vanillin	Merck	1.31

* From reference 16.

HPLC System

RPLC studies were performed with the following columns: Polymeric reverse phase (Hamilton PRP-1) 150x4.1 mm, 5 μm, (Hamilton, Reno, U.S.A.), deactivated octyl silica (Supelco LC-8-DB) 150x4.6 mm, 5 μm (Supelco, Bellefonte, U.S.A) , octyl silica (Kromasil C-8) 250x4.6 mm, 5 μm, (Eka Nobel, Sweden), deactivated octadecyl silica (Supelco LC-18-DB) 150x4.6 mm, 5 μm, (Supelco, Bellefonte, U.S.A.). The mobile phase contained typically acetonitrile:ammonium acetate 100 mM): triethylamine (TEA) (65: 35: 0.05), and pH of the aqueous phase was adjusted to 6.4 with concentrated acetic acid. In one experiment, methanol:ammonium acetate:TEA (80: 20: 0.05) was used as a mobile phase.

The HPLC apparatus consisted of Beckman (Altex) 210 A injector, Beckman 116 M solvent delivery system, Beckman 165 variable wavelength detector (Beckman Instruments Inc., Berkeley CA U.S.A.). Chromatograms were recorded with a Scintag 3122 (Scintag, Switzerland) strip and chart recorder. The flow rate was 1.0 mL/min, and the wavelenght of the UV detector varied between 230 and 255 nm depending on the compound chromatographed.

RPTLC System

As an aid in method development, reverse phase thin layer chromatography (RPTLC) was performed with 100x100 mm octyl bonded plates (Merck, Darmstadt, Germany). Samples were applied with Camag Linomat IV sample application system (Camag, Muttenz, Switzerland). Acetonitrile or methanol were tried as organic modifiers with a same kind of buffer as in HPLC, supplemented with TEA. The spots were visualized in UV light at 254 nm.

Calculation of Retention Parameters

Retention volumes (V_R) of compounds examined were measured from chromatograms. The samples were run as triplicates. The column dead volume (Vm) was determined by using sodium nitroprusside. The capacity factors of the compounds were calculated from the equation $k = V_R\text{-}Vm/Vm$.[15] Logarithms of capacity factors (log k) were plotted against log P - values of the reference compounds, the latter values being obtained from literature.[16] The correlations and graphs were produced with a Cricket Graph program of a Macintosh Plus PC. The R_m-values in RPTLC were calculated from the equation $R_m = \log (1/R_f\text{-}1)$.[17]

RESULTS AND DISCUSSION

Stationary Phase Effects

The earlier HPLC methods for toremifene and its metabolites have utilized reverse phase C_{18} columns[7,8] or a cyano column.[9] Because of the quite high pH values of the mobile phases used in those investigations that have been described, e.g. pH 6.4 - 8, we first tried a polymeric reverse phase (PRP-1) column. The reason for this was that polymeric phases stand a high pH (2-13)[18] and also, that a PRP-1 phase has been recently used succesfully for lipophilic compounds.[19] However, for triphenylethylenes the PRP-1 column, length being 150 mm and particle size 5 μm, proved not to be satisfactory at all, even though using a high portion of acetonitrile in the mobile phase. Most of the peaks were not sharp enough, although they were symmetric.

The next column evaluated was Supelco LC-18-DB, 150x4.6 mm, particle size 5 μm. As expected, the octadecyl silane phase causes much retention for the most lipophilic triphenyethylenes, like toremifene. This is also an advantage in separations from biological samples, if good peak shapes are obtained, and the speed of analysis can be improved by higher flow rates.[7,8]

Capacity factors obtained with this C_{18} column are presented in Table 3. For an unknown reason, one compound, FC-1530 B (10, Table 1) could not be chromatographed with this column at all.

A deactivated octyl silane column, Supelco LC-8-DB, 150x4.6 mm, 5 μm particle size, produced the best retention behavior for the whole series of triphenylethylenes studied. The capacity factors were markedly smaller than for the corresponding C_{18} column (Table 1) and therefore, this C_8 column seems to better suit our studies on log k values describing lipophilicity, and also for studies of synthetic product purity. Peak shapes were also more satisfactory when using Supelco C_8 compared with the C_{18} one.

Kromasil C_8 250x4.6 mm, 5 μm particle size, was included in this study being a cheap column, but producing good results in other HPLC studies . This column is not pronounced to be deactivated, but it seems to be tightly packed or properly end-capped, because the peaks had good shapes. This column was quite near the quality of Supelco LC-8-DB when concerning the aims of our studies. It is true, that this column is longer than the corresponding Supelco. The capacity factors of triphenylethylenes are presented in Table 3.

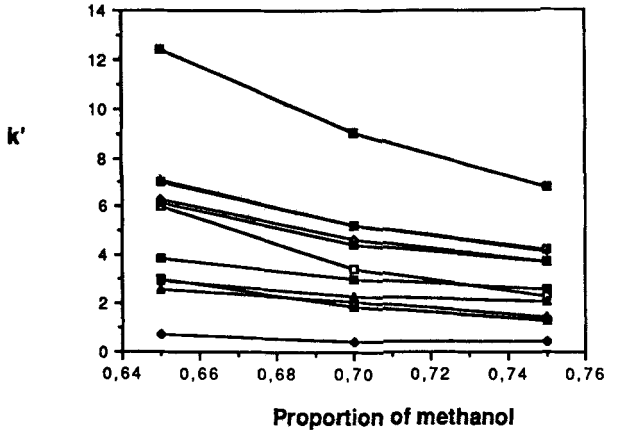


Figure 1. Capacity Factors of twelve thriphenylethylene derivatives as a function of percentage of acetonitrile in the mobile phase. Column: Supelco C_8.

Table 3

Capacity Factors (k) for Triphenylethylene Derivatives (1-12) for Different Silica Based RP Columns

	Column		
Compound	**Supelco C_{18}**	**Supelco C_8**	**Kromasil C_8**
1.	9.31	5.55	8.58
2.	0.64	0.69	0.79
3.	4.42	2.48	5.37
4.	9.23	5.02	7.16
5.	10.92	5.52	8.53
6.	5.04	3.09	3.52
7.	11.00	4.83	7.26
8.	3.55	2.41	2.79
9.	2.31	2.11	2.32
10.	*)	9.64	*)
11.	11.08	5.51	8.63
12	8.84	4.91	7.49

*) Could not be chromatographed in given conditions.

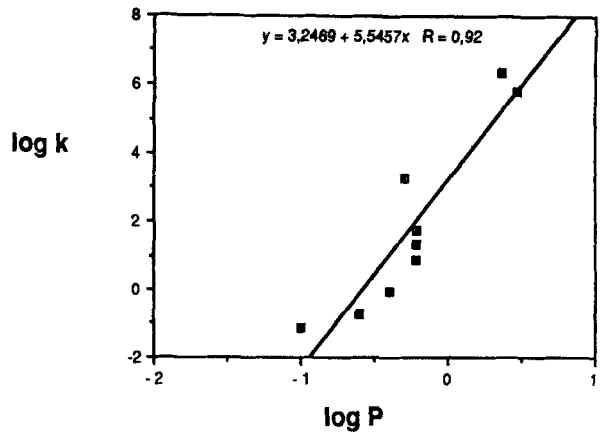


Figure 2. Correlation of log k and log P for reference compounds. Column: Hamilton PRP-1.

Mobile Phase Effects

Because of the highly lipophilic character of toremifene (log P= 6.35), the composition of mobile phase was constructed to contain a high portion of organic modifier. This approach has been used for lipophilic compounds.[19] Toremifene and some of its main metabolites, some of them included in this study, have been analysed by HPLC from biological samples using acetonitrile:ammonium acetate: triethylamine (TEA) as mobile phase.[8]

Other mobile phases that have been used in HPLC of toremifene and its metabolites have been: methanol:water (93:7) with 0.1 % TEA[7] and methanol: 0.1 M ammonium acetate (pH 8) (70:30). The mobile phase composition containing 65 % acetonitrile,[8] was first tested with RPTLC and it was then moved to HPLC. When trying RPTLC with MeOH:buffer with lower portions than 70 % of MeOH, toremifene and its analogues had far too much retention.

The mobile phase composition, according to Webster et al.,[8] was chosen to for this work because the main aims were to test columns and capacity factors for a series of triphenylethylenes. When varying the percentage of acetonitrile in the mobile phase (deactivated octyl silane column), the capacity factors were clearly affected (Fig 1). The PRP-1 column showed the kind of tendency as mentioned before, e.g. no satisfactory conditions were found for that polymeric phase. The diversity was found in lipophilicity studies as seen later.

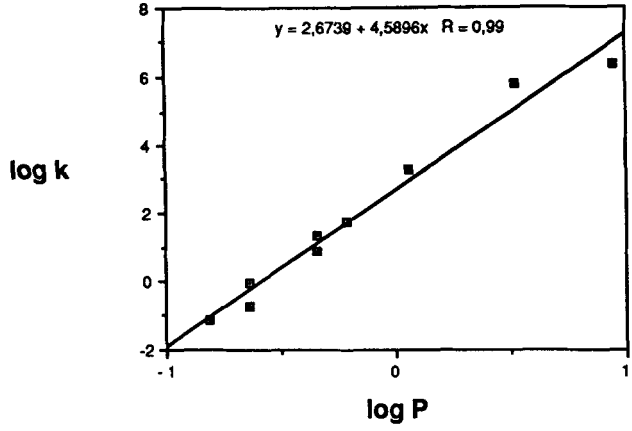


Figure 3. Correlation of log k and log P for reference compounds. Column: Supelco C_8.

Evaluation of Lipophilicity

In addition to method development, RPTLC was aimed to be used as a method for studying the lipophilicity of triphenylethylenes. RPTLC is a simple, rapid and low-cost method for such purposes, but accuracy is poor, especially when a UV scanner is not available. Even then, its applicability is not always satisfactory, owing to the unaccurate determination of the solvent front.[20]

Polymeric reverse phase (PRP-1) column is stabile in extreme pH conditions, and also applicable to highly lipophilic compounds when evaluating lipophilicity,[19,21] but in this case the peak shapes were unacceptable for some important compounds. Also, correlations between log k and log P values when using reference compounds were markedly worse than those when using silica based stationary phases (Fig 2). These correlations could not be improved by changing the percentage of acetonitrile in the mobile phase.

Silica based stationary phases gave satisfactory correlations for reference compounds, when log k values from HPLC and corresponding log P values from literature were subjected to linear regression (Table 2). Figures 3-5 present regression plots of those correlations obtained with Supelco C_8, Supelco C_{18} and Kromasil C_8.

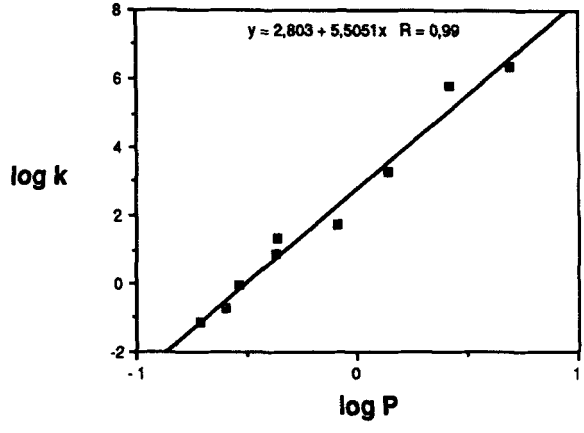


Figure 4. Correlation of log k and log P for reference compounds. Column: Supelco C_{18}.

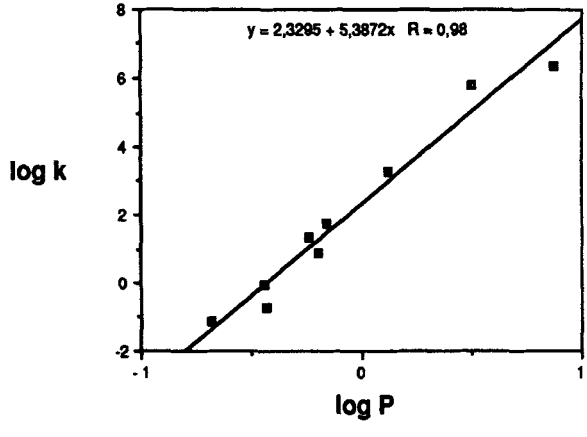


Figure 5. Correlation of log k and log P for reference compounds. Column: Kromasil C_8.

The results indicate, that these columns, preferably the C_8 coated ones, are suitable for lipophilicity studies for triphenylethylenes and related compounds even when a high portion of acetonitrile is used. The definitive evidence

should be obtained from comparison of triphenylethylenes themselves, by using a shake flask method and HPLC, but the very high lipophilicities of those compounds render this approach impossible.

One alternative to get parameters for a comparative study should be the use of calculation of log P by a computer, but we do not have such systems available. It seems obvious that lipophilicity parameters can be used for QSAR studies according to Braumann,[12] e.g. reverse phase columns simulate biological bilayers well, although some doubts have been presented and also new stationary phases have been introduced.[22]

In addition, mobile phase used here, containing acetonitrile as organic modifier, according to preliminary data seems to be suitable also for lipophilicity studies. This tendency has been noticed in studies performed with another classes of compounds,[19,23] in spite of doubtful findings for some series of compounds.[24] It is obvious, that mobile phase containing acetonitrile:ammonium acetate (65:35) with triethylamine as the amine modifier at pH 6.4, is suitable for RPLC purity studies of new triphylethylenes when using UV detector. Accordingly, it is possible to use this combination of mobile phase without the amine as an eluent for thermospray LC-MS of these compounds, at least, when using a deactivated RP column like Supelco LC-8-DB.[25,26]

CONCLUSIONS

Octyl silane (C_8) stationary phases are preferred for RPLC of triphenylethylenes, which are highly lipophilic compounds. Octadecyl chains cause too long a retention and also peak shapes are not satisfactory. In most cases, polymeric reverse phase column does is suitable for these molecules. A deactivated Supelco C_8 and Kromasil C_8 phase have almost equally good properties in retention of derivatives in question. A 150 mm long Supelco produces slightly better peak shapes than a 250 mm long Kromasil, but the latter one is markedly cheaper. The mobile phase should contain a large portion of organic modifier, and in this case acetonitrile (65 %) is the solvent of choice. With ammonium acetate (0.1 M) as a buffering salt, this system meets the requirements of thermospray LC-MS solvent, and in forthcoming studies that methodology will be applied. These RPLC conditions qive the possibility to go on to lipophilicity studies of these and new triphenylethylenes, in order to make use of retention data for quantitative structure relationship studies performed with CoMFA (Comparative Molecular Field Analysis). The shake flask method is not applicable because of the fairly high lipophilicities of these compounds, and mostly, for the same reason, the more conventional methanol/water mobile phase of RPLC combination is not preferable for these studies.

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