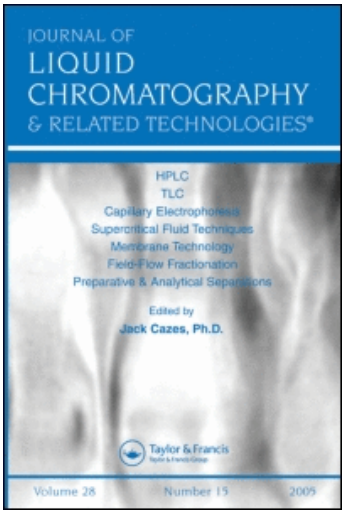

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CORRELATION OF TLC, HPTLC AND PMD RESULTS AND THEIR TRANSFER TO HPLC SYSTEMS

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ABSTRACT

The correlation of the results of five thin-layer chromatographic techniques and their transfer to high performance liquid chromatography are reported. The data obtained indicate that high performance thin-layer chromatographic results are faithfully reproduced by high performance liquid chromatography. Although plates were not activated, and mixed solvents were used, no major differences were observed between thin layer and high performance liquid chromatography.

INTRODUCTION

Thin layer chromatography (TLC) has developed into one of the more powerful research tools and it is in routine use in many fields for separation, and qualitative and quantitative analyses. The success of TLC can be attributed

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to its sensitivity, selectivity, ease of operation and low cost (1). On the other hand, another separation technique, high performance liquid chromatography (HPLC) is enjoying a rapid growth and acceptance. As HPLC became known, many researchers expected that it would replace TLC in many applications. Today we know that both chromatographic methods will continue to exist each in its own right, and that they are well suited to complement each other (2). Both HPLC and TLC require a search for both a stationary and a mobile phase. The proper conditions are found by trial and error, based on the properties of the sample to be separated, the stationary phase (silica gel, reversed phase, ion exchange, etc...) and the mobile phase. This may be a time consuming process. However, the time for selection of suitable experimental conditions for both methods could be reduced drastically could the mobile phase be selected easily and were the conditions of one method (HPLC or TLC) transferrable to the other.

Recently, two TLC techniques, the Vario-KS chamber (3) and the Selecta Sol system (4), were introduced with the capability of simultaneous plate development in more than one solvent system. This added dimension of rapid selection of the solvent system makes TLC the method of choice for selecting the mobile phase for both TLC and HPLC.

Coq, et al. (5) studied the possibility of establishing a simple relationship between the retention of the solutes in column chromatography and their R_f values in TLC. Their results show the transfer of many systems under controlled experimental conditions. In another study (6), a comparison of HPLC and TLC data obtained with various types of silica showed that the graphical correlations of column and TLC data are satisfactory, confirming the usefulness of TLC as a reconnaisance technique for column experiments. It was also reported (6) that when different adsorbents of the same general type were used with different composition of the mobile phase, the transfer of the results from TLC to HPLC were satisfactory when plates were pre-conditioned and tanks were saturated. The separation of dansyl derivatives of polyamines and related compounds were first investigated by TLC, and then the systems which seemed promising were then

adopted by HPLC (7). Majors (8) reported the separation of six azo dyes on TLC and HPLC on silica gel using methylene chloride:hexane (10:90). As expected, the order of elution of the six dyes was similar to the TLC plate.

The introduction of high performance TLC (HPTLC) (9) as a nano-separation technique has the advantages of speed and screening, not only of samples, but also of experimental conditions, such as controlled humidity, adsorbent, solvent system and solvent flow. These controlled conditions make HPTLC compatible with HPLC. Qualitative HPTLC can be used for routine analysis and as a rapid preliminary technique for HPLC (10), however, HPTLC data can be transferred to HPLC system only if the phases are the same (11). Janchen (2) reported that development in the unsaturated sandwich configuration is obviously not suitable for the TLC-HPLC transfer.

This study reports the correlation of TLC results and HPLC using five different TLC techniques. Four groups of compounds, which are of interest in cancer research, were tested. Two adsorbents, silica gel and reversed phase, were selected based on previous experience. These correlations were made using commercially-available and not specially prepared plates and columns, in order to make the study more relevant to available laboratory materials which are used routinely by the worker who is more interested in separation than in the theory of the subject.

EXPERIMENTAL

Apparatus. The following apparatus were utilized in this study.

TLC: Vario-KS chamber (Camag); HPTLC U-chamber (Camag); Programmed Multiple Development (Regis Chemical Co.); Selecta Sol (Schleicher and Schuell); and a viewing cabinet with long (366 nm) and short (254 nm) UV lamps (Brinkman). Standard glass tanks were used for plate development.

HPLC: The HPLC unit consisted of a Constametric II pump (LDC), dual channel UV detector 254 nm and 280 nm (Spectra Physics), a high pressure six port sampling valve fitted with an injection syringe (Model VIS, Glenco) and a strip chart recorder (Omniscribe, Houston Inst.).

Reagents and Materials. All solvents were glass-distilled (Burdick and Jackson). TLC and HPTLC precoated plates were EM silica gel 60 with and without fluorescent indicator. Reversed phase precoated plates were EM silica gel 60 silanized without fluorescent indicator.

The HPLC columns used were microparticle silica Partisil-10 (Whatman, Inc.) and bonded reverse phase, 6-8 μ-Zorbax ODS (Dupont). The compounds selected were: 1-hydroxy-2-acetylaminofluorene (1-OH-2-FAA), 3-hydroxy-2-acetylaminofluorene (3-OH-2-FAA), 5-hydroxy-2-acetylaminofluorene (5-OH-2-FAA), and 7-hydroxy-2-acetylaminofluorene (7-OH-2-FAA). These chemicals were kindly supplied by Dr. H. R. Gutman, Cancer Research Laboratory, Veterans Administration Hospital, Minneapolis, MN. Adenine and 9-methyladenine (9-MeA) (Sigma Chemicals), N^6-MeA and N^6-diMeA-9-ethyl A (Cyclo Chemicals). 7,12-dimethylbenzanthracene (DMBA), DMBA-5,6-diol, 7-hydroxymethyl-12-methylbenz(a)anthracene (7-OH-Me-12-MBA) (kindly supplied by Dr. R. Shephard, FCRC), P-nitrobenzyl esters of lithocholic acid, isolithocholic acid, 3-keto-5β-cholanic acid and 5β-cholanic acid (kindly supplied by Dr. M. Kelsey, FCRC). Compounds were used without purification.

Procedure: Solutions of the acetylaminofluorenes and the adenines were made in methanol; those of DMBA and its analogs in ethanol; and bile acid esters in 50% chloroform:methanol. Solutions were spotted on TLC plates using Drummond micropipetts. For HPTLC plates a special micro-syringe, supplied with the unit, was used. Plates

were used without preactivation but were kept in closed boxes in a dry place. Tanks were allowed to equilibrate with the solvent vapors but were not lined with filter paper. After development and drying in the hood, spots were viewed under UV light (365 nm for DMBA and its analogs and 254 nm for the others). The solvent system which seemed promising in classical TLC, and the Vario-KS Chamber was transferred to HPTLC, PMD and HPLC. HPLC was operated at ambient temperature. Other experimental conditions are listed in the legends to the figures. Selecta Sol was used for the adenine bases only. All HPTLC plates were developed using the U-chamber unless stated otherwise.

It was decided to use the same type of silica gel from the same manufacturer on both TLC and HPTLC (silica gel 60) in order to eliminate any errors due to coating, binder, adsorbent size and activation. Also, plates from the same box were used for the same group of compounds, except HPTLC, where nano-TLC plates were used.

The plates were spotted as quickly as possible to minimize exposure to the atmosphere since humidity can affect TLC results and their transfer to HPLC systems (2). Conditions that gave good separation by TLC were transferred to HPLC.

RESULTS AND DISCUSSION

To assess the transfer of TLC data to HPLC systems, silica gel and silanized silica gel were used to separate four groups of compounds: (a) adenine, 9-methyladenine, N^6-methyladenine and N^6,N^6-dimethyl-9-ethyladenine; (b) hydroxy acetylaminofluorenes (1-OH-2-FAA, 3-OH-2-FAA, 5-OH-2-FAA and 7-OH-2-FAA); (c) p-nitrobenzyl esters of bile acid metabolites (lithocholic acid, isolithocholic acid, 3-keto-5β-cholanic acid and 5β-cholanic acid); and (d) dimethylbenzanthracene metabolites (7,12-DMBA, DMBA-5,6-diol and 7-OH-Me-12-MBA).

Five different TLC techniques were used. The Vario-KS chamber, the HPTLC U-chamber, Programmed Multiple Development (PMD), the Selecta Sol, and classical TLC in a tank (1). These five were selected for the following reasons. Classical TLC gives a saturated atmosphere and is very widely used. The Vario-KS chamber has the advantage of speed of solvent selection under controlled atmosphere, and five solvent systems can be tested simultaneously. PMD requires weaker solvents relative to classical TLC; so does HPLC. Parris (12) observed that compounds having high R_f values in classical TLC systems require weaker solvents by HPLC; and those with low R_f values require stronger solvents. PMD is a sandwich type system. It was suggested by Janchen (2) that sandwich type TLC is not suitable for TLC-HPLC data transfer. HPTLC uses plates with a narrower particle size distribution and a thinner coating with a layer density comparable with a well-packed column. It also has a controlled atmosphere and the samples may be injected into the solvent stream in the same way as in HPLC. Selecta Sol is similar in concept to the Vario-KS chamber and gives circular development in up to 16 solvent systems. Both HPTLC and Selecta Sol systems develop extremely quickly, but HPTLC is superior in design concepts and allows better control of experimental conditions.

The results obtained with the different TLC modes listed above, and HPLC are given in Table I. The results indicate that (a) separations obtained by TLC on silica gel plates can be reproduced by HPLC on silica gel columns; (b) it was harder to reproduce HPLC separation on reversed phase by TLC because the solvent mixture contained a high percentage of water which caused the adsorbent to come off the plate; (c) HPTLC conditions were transferrable to HPLC except for the adenine bases in (10:90) MeOH:CH_2Cl_2 (13); and (d) in order to achieve complete separation of p-nitrobenzyl esters of bile acids in all modes used, it was necessary to change the strength of the solvent mixture. PMD gave two and four spots in 2.5% and 10% isopropanol:hexane respectively, while HPLC gave four peaks when 1-3% isopropanol was used (14).

The HPLC resolution of a mixture of 1, 3, 5 and 7-OH-2-FAA on reversed phase column (Figure 1) was better than that on a silica gel column (Figure 2).

Table I. Comparison of TLC, V-KS, PMD, HPTLC, and HPLC Results.

COMPOUND, SOLVENT AND ADSORBENT	TLC # OF SPOTS	V-KS # OF SPOTS	PMD # OF SPOTS	HPTLC # OF RINGS	HPLC # OF PEAKS
(a) Adenines					
(1) 10% MeOH:$CHCl_3$, Silica Gel	4	4	3	4	4
(2) 10% MeOH:CH_2Cl_2, Silica Gel	3	3	3	4	3
(b) Bile Acids					
(1) 2.5% Isopropanol:Hexane, Silica Gel	4	4	4**	4	4
(c) FAA					
(1) 40% EtOAc:Hexane, Silica Gel	4	4	4	4	4
(2) 35% CH_3CN:H_2O, Reversed Phase	NA*	NA*	NA*	NA*	4
(d) DMBA					
(1) 85% CH_3CN:H_2O, Reversed Phase	2	2	NA*	3	3

*NA = NOT APPLICABLE

**10% ISOPROPANOL:HEXANE

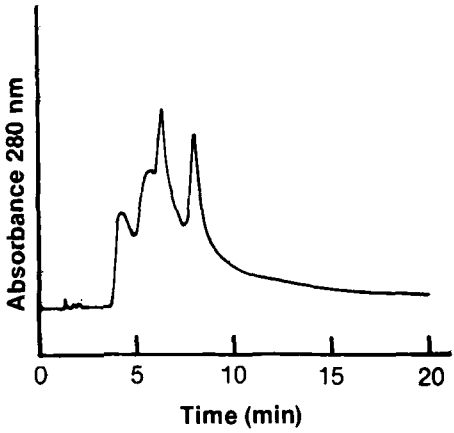

Fig 1. HPLC chromatogram of the mixture of 1, 3, 5 and 7-hydroxy-2-FAA
Conditions: Column, Partisil-10 25 cm x 4.6 mm; mobile phase, 60% ethylacetate and UV, 280 nm.

Although it is not mentioned in Table I, Selecta Sol resolved the four adenine bases on both HPTLC plates and 0.25 mm silica gel 60 plates. The results matched those obtained with the U-chamber (Figure 3). Since the U-chamber has controlled solvent flow, while the Selecta Sol does not, it was decided to omit the Selecta Sol. No difference was observed in separation or resolution when the p-nitrobenzyl ester of bile acids mixture was spotted or injected into the solvent stream when the U-chamber was used (Figure 4, 5). In this study it was decided to include two compounds, 9-MeA and N^6-MeA, which gave close R_f values of 0.46 and 0.47 (13) to see if HPLC gives better resolution. The HPLC results (Figure 6) matched those of TLC.

Parris (12) discussed the transfer of TLC data to HPLC and concluded that although, in principle, both methods might be considered as two ways of performing the same type of separation, considerable caution should be exercised when transferring TLC methods to a modern liquid - solid column chromatographic system.

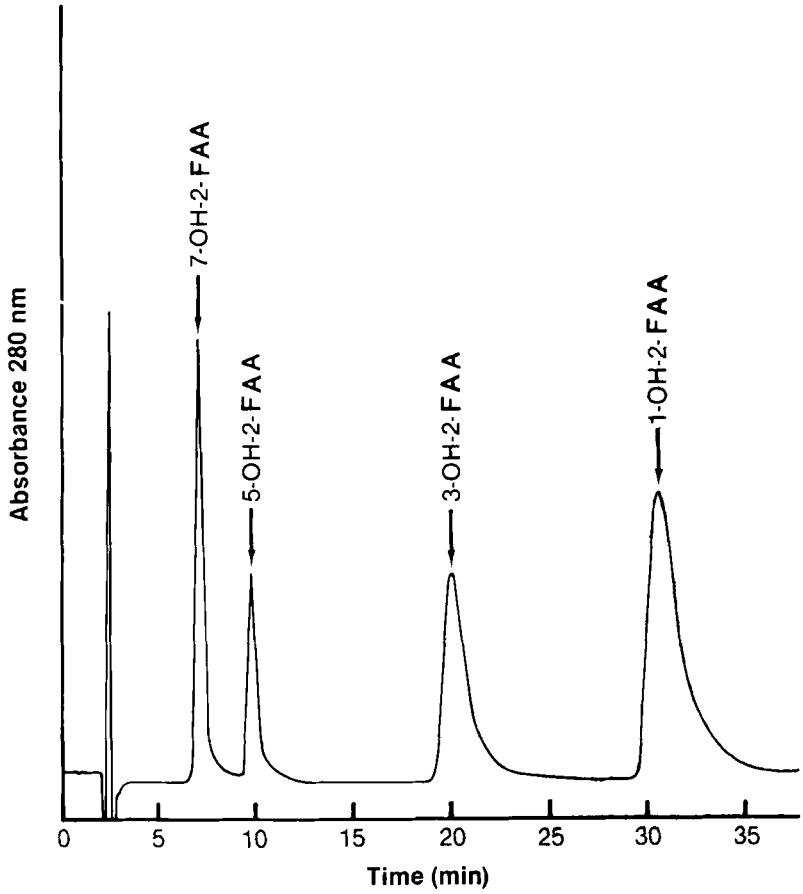

Fig 2. HPLC chromatogram of the mixture of 1, 3, 5 and 7-hydroxy-2-FAA Conditions: column, Zorbax ODS 23 cm x 4.5 mm; mobile phase, 35% acetonitrile in water: UV, 280 nm; flow rate, 1 ml/min.; chart speed, 0.2 in/min.

The reasons for this discrepancy are, firstly, that in most cases a TLC plate is used in a highly activated form whereas a LC column has been deactivated to some extent by the passage of the mobile phase through the column prior to injection of the sample. Secondly, with few exceptions, the type of

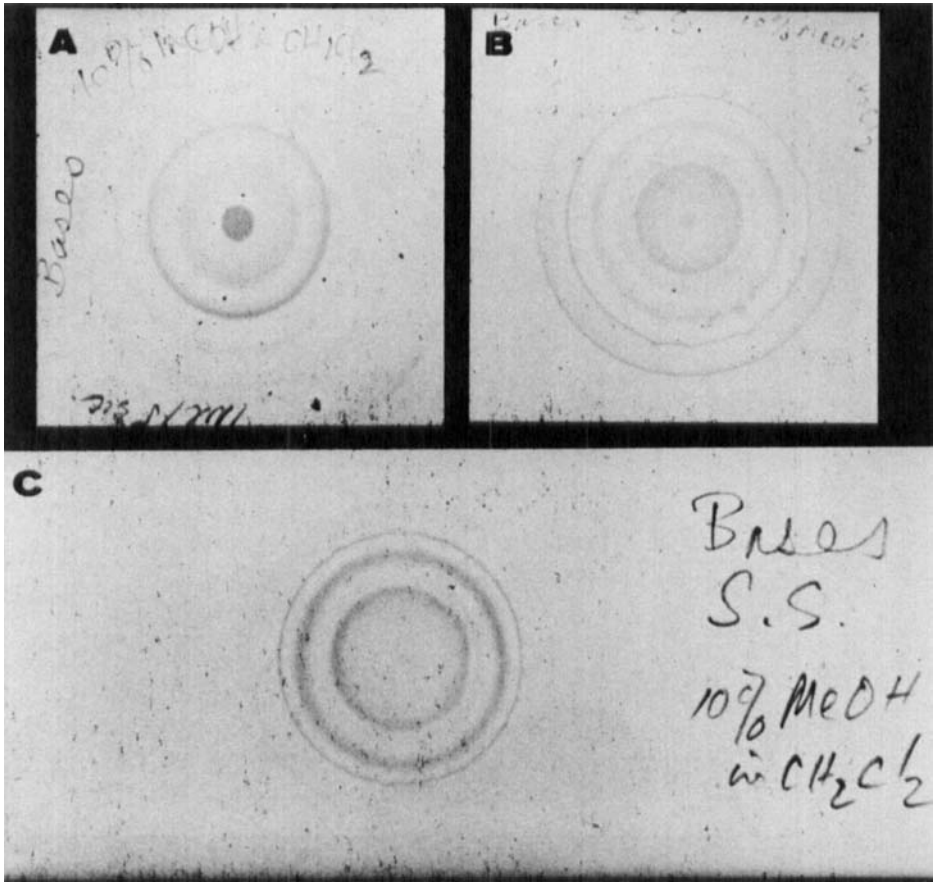

Fig 3. Chromatogram of adenine, 9-MeA, N^6-MeA and N^6-diMe-9-Ethyl A on silica gel 60 plate in MeOH:CH_2Cl_2 (10:90) on HPTLC plates using the U-Chamber (A) and Selecta Sol (B) and on conventional TLC plates by Selecta Sol and the same solvent system (C)

adsorbent used for TLC varies considerable in particle size distribution, surface area and pore size relative to the LC counterpart.

Coq, et al. (5) observed,using adsorption chromatography, that it is possible to transfer TLC data to column chromatography under controlled experimental conditions. Adsorbents and activity of adsorbents must be the same.

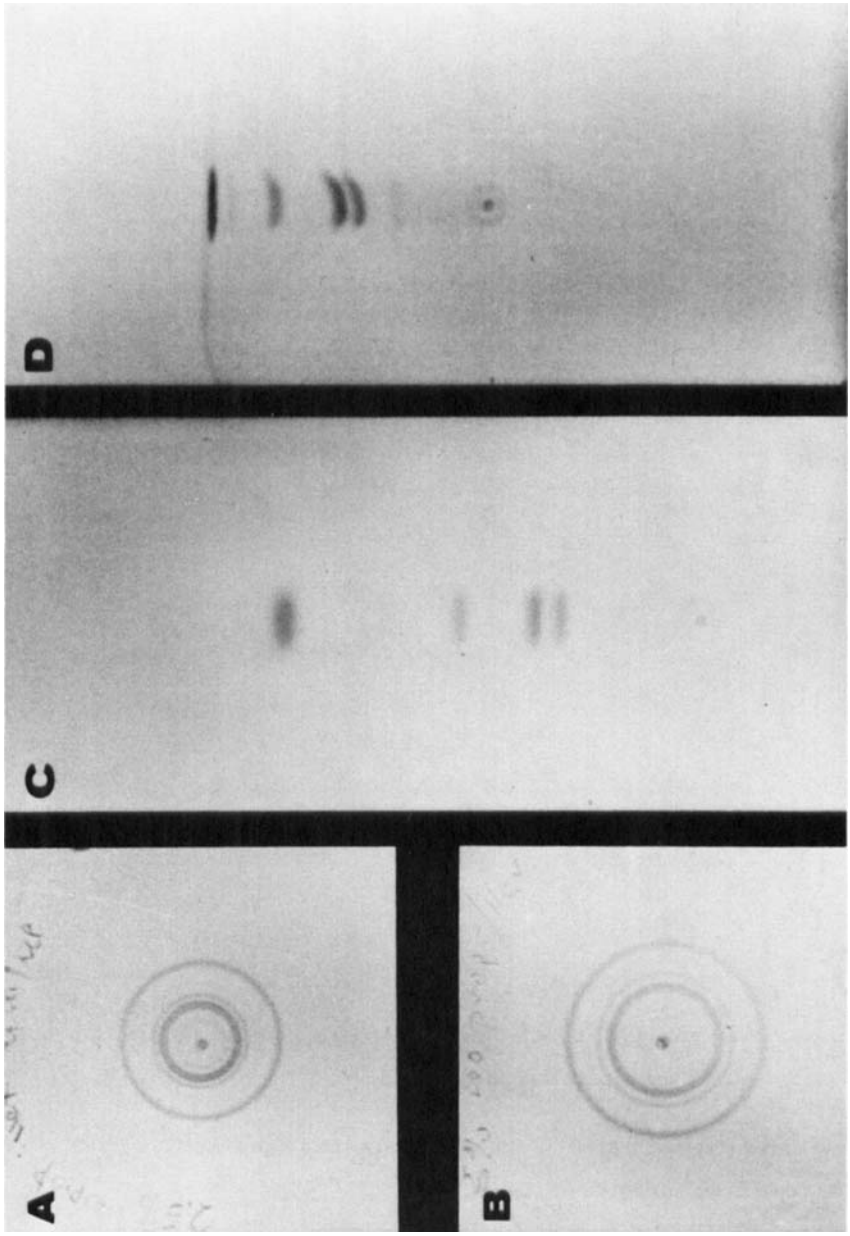

FIG. 4. Chromatogram of p-nitrobenzyl esters of bile acid metabolites (see text) developed on silica gel plates in (A) (2.5:97.5) isopropyl alcohol:hexane using HPTLC plates and U-chamber; (B) same as A but (5:95) isopropyl alcohol:hexane; (C) conventional TLC solvent system same as in (B); and (D) by PMD in 10:90 isopropyl alcohol:hexane.

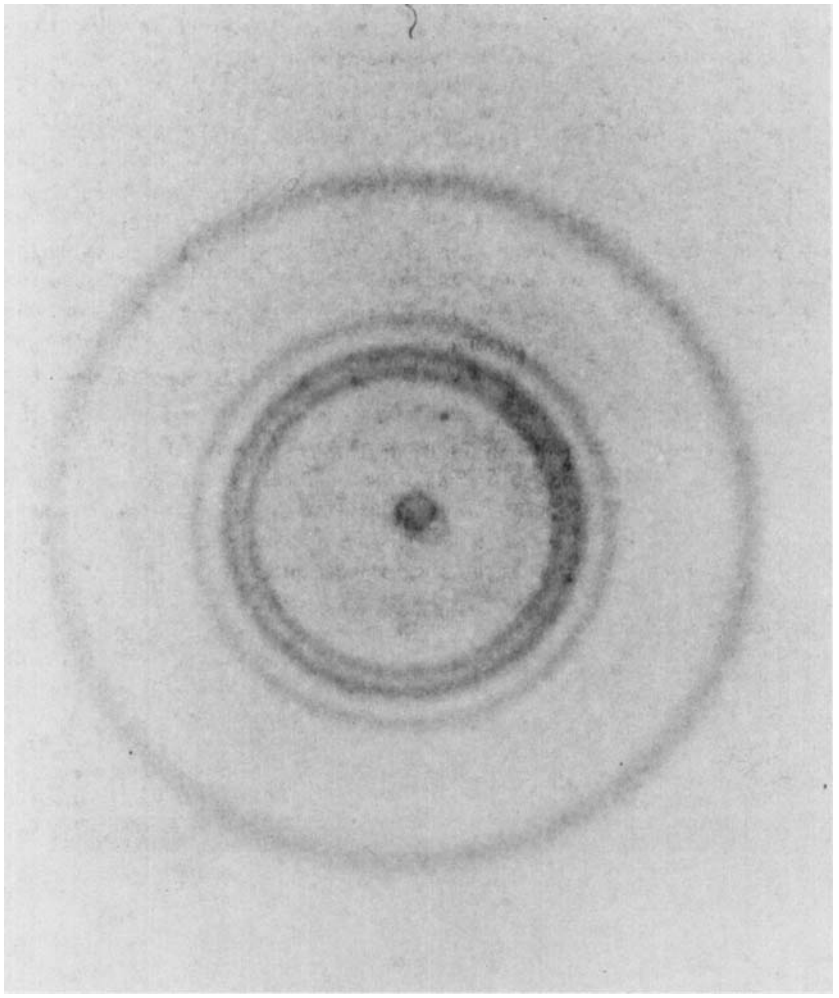

Fig 5. Same as Fig 4A, except that the sample was injected into the solvent stream and not spotted on the plate.

They (5) also reported that it is far more difficult to reproduce TLC results by HPLC when mixed solvents are used.

There are three TLC characteristics which might affect HPLC separations. One of these has been mentioned earlier (15). It is the activity of the plate

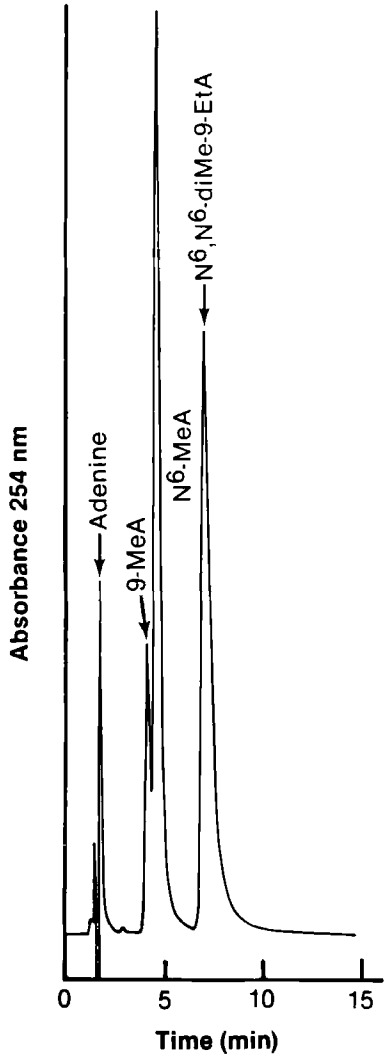

Fig 6. HPLC Chromatogram of the mixture of adenine bases. Conditions: column, Partisil-10 25 cm x 4.6 mm; mobile phase, 10% methanol in chloroform, UV, 254 nm; flow rate, 2 ml/min; chart speed, 0.2 in/min.

which might vary, since the plate may be exposed to the atmosphere while the column is not. However, these differences can be accounted for by changing the strength of the mobile phase or by activating the plate. Another problem is solvent demixing which usually occurs with mixtures of solvents in which the strong component is less than 5%. For detailed discussion see Snyder (16). A third consideration is the evaporation of solvent during development, for this affects the K' (the capacity factor). This can be minimized by using a sandwich or saturated chamber.

Kirkland and Snyder (15) also concluded that in the transfer of TLC results to HPLC systems it is important to have the same adsorbent and the same solvent. When the same adsorbent and solvent are used in each case, the capacity factor (K') values in column chromatography can be predicted from TLC R_f values by the following equation $K' = (1-R_f)/R_f$. A comparison of K' calculated from R_f values using TLC and HPTLC, and from R_f values is shown (Table II). The K' values obtained by both techniques are in agreement if we take into consideration that the experimental conditions were not controlled.

CONCLUSION

Our results indicate that the transfer of TLC results to HPLC system is possible even under uncontrolled experimental conditions. Although plates were not activated, no major differences were observed between both techniques. Also, the results of mixed solvents were transferred from one system to the other with, in certain cases, minor adjustment in the strength of the solvent. Using different modes of TLC, it was observed that sandwich type chambers may be used for selecting HPLC mobile phase. The use of PMD may pose a problem when the solvent mixture used contains water. Also, it was not possible to use reversed phase plates when (35:65) acetonitrile:water was used because the adsorbent came off the plate.

Although HPTLC gave equal or better separations than the other TLC modes used, it is clear that classical TLC in a tank is adequate for selecting the

Table II. Comparison of K'_{TLC} and K'_{HPTLC} with K'_{HPLC} values.

	TLC	HPTLC	HPLC
	K'	K'	K'
Bile Acids[a]			
5-β	0.45	0.59	0.2
3-Keto	1.5	0.85	1.32
I-LA	2.57	1.13	3.72
LA	3.16	2.57	5.72
Adenines			
N^6,N^6-diMe-9EtA	0.11	0.27	0.5
N^6-MeA	1.13	0.79	2.33
9-MeA	1.17	1.00	2.83
Adenine	3.17	1.86	4.83
FAA			
7-OH-2-FAA	0.59	0.54	2.23
5-OH-2-FAA	0.89	0.72	3.31
3-OH-2-FAA	1.22	1.27	3.85
1-OH-2-FAA	2.03	1.63	5.15

[a] 5-β= 5β-cholanic acid
3-Keto= 3-Keto-5β-cholanic acid
I-LA= isolithocholic acid
LA= lithocholic acid

HPLC conditions. Therefore, it is recommended that TLC be used for selecting these conditions before starting an experiment due to the amount of time and materials saved.

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