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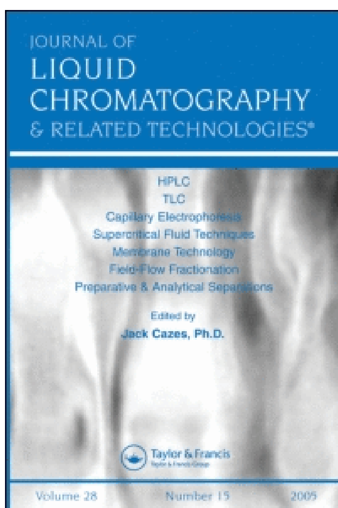

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SOLVENT EFFECTS ON THE PRECISION OF SAMPLE APPLICATION IN QUANTITATIVE THIN-LAYER CHROMATOGRAPHY

by

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SUMMARY

The use of non-polar solvents for sample application in quantitative thin-layer chromatography (TLC) was shown to have a marked effect on the precision of the analytical technique when polar compounds were applied to silica gel plates. Increasing the polarity of the application solvent allowed competitive binding of the solvent molecules to silica, thereby deactivating the silica at the origin. Studies showed that the coefficient of variation for TLC assays were substantially reduced by altering the polarity of the application solvent.

INTRODUCTION

During the quantitative thin-layer analysis of compounds isolated from biological fluids or tissues, it is often necessary to concentrate the organic extract when assaying low concentrations of drugs. This is usually accomplished by evaporating a portion of the organic extract to dryness and redissolving the residue with a non-polar solvent suitable for application to a TLC plate. A common practice is to reconstitute with the least polar solvent in which the drug is soluble[1]. This approach is well-founded since the interfering materials are usually reduced, and the ease of solvent evaporation from the surface of the plate during sample application is enhanced. However, it has been observed in our laboratory that strict adherence to this principle can, in some instances, lead to a loss in assay precision. For example, in developing a method for the measurement of 3-bromocinnamic acid (IV) in plasma, the mean recovery was satisfactory, but the precision was poor with a 15% coefficient of variation (C.V.). In the initial sample preparation scheme, residues from extracts were

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redissolved in chloroform, but subsequent investigation established that by redissolving the residue in a methanol-chloroform (15:85) solution v/v, the precision of the assay could be improved dramatically. This same phenomenon was observed with other compounds. Since it does not appear that this aspect of sample preparation has been previously addressed, additional studies were conducted. The results of this work are now reported.

PROCEDURE

A) TLC

Solvents used for these experiments were from Burdick & Jackson. Silica gel 60 plates (20 x 20 cm, EM Laboratories) were scored into 20 x 1 cm channels and were mechanically spotted (18 channels) with an A.I.S. Multispotter using 100 μl Unimetrics gas/liquid syringes. The compounds were spotted (100 μl volumes) with drug concentrations in the 1-5 ng/μl range) from standard solutions prepared with 100% chloroform or a methanol-chloroform (15:85) mixture. A gentle stream of warm air was blown across the surface of the plate to aid solvent evaporation, and the plates were developed in appropriate systems. In a typical experiment one-half of the channels on a single plate were spotted with a chloroform solution of a drug and the remaining channels were spotted with a methanol-chloroform solution of the same compound. Quantitation was accomplished using a Schoeffel SD 3000 spectrodensitometer in the absorbance or fluorescence mode, while peak areas were integrated with an Autolab minigrator and recorded.

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B) HPLC

Samples were injected (20-50 μl) with a 100 μl S.G.E. syringe into a Waters Associates, model ALC/GPC-204 high pressure liquid chromatograph (HPLC) equipped with a micro-Porasil column. The mobile phase [(3-7%) isopropanol-chloroform] was pumped at 1 ml/min. Absorbance at 254 nm was monitored and peak-height measurements obtained from a Linear Instruments Corp. strip-chart recorder. For repetitive injections the syringe was washed sequentially with dilute acid, methanol, chloroform and air-dried before each injection.

RESULTS

When six compounds (Table I) were spotted in chloroform solutions on silica gel plates, developed in appropriate solvents and quantitated, the reproducibility of the peak areas determined for three of the compounds, 3-bromocinnamide (I), phenacetin (III) and theophylline (VI), was excellent (C.V. ±1.2%). However, for the other three compounds, 2[2-(aminomethyl)-phenylthio]benzylalcohol (II), 3-bromocinnamic acid (IV) and aspirin (V), the reproducibility was poor (C.V. > ±15%). When methanol (15%) was added to the same volumetric flask containing compounds II, IV and V and the samples respotted, the precision improved with the C.V. lowered to 4% or less. It might be noted

TABLE I

EFFECT OF THE SPOTTING SOLVENT ON ASSAY PRECISION

COMPOUND	SPOTTING SOLVENT	COEFFICIENT OF VARIATION[b]
3-Bromocinnamamide (I)	A[a]	± 1.2%
2[2-(Aminomethyl)-phenylthio]benzyl alcohol·HCl (II)	A	±23%
	B	± 4%
Phenacetin (III)	A	± 1.3%
3-Bromocinnamic Acid (IV)	A	±17%
	B	± 2.8%
Aspirin (V)	A	±28%
	B	± 2.7%
Theophylline (VI)	A	± 1.2%

[a] Solvents used for application of samples to plates: A, chloroform; B, methanol-chloroform (15:85).

[b] Repetitive applications were n = 12.

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that although the spotting solvent was not mentioned, our results for theophylline agree with previous data on reproducibility (±1.2% C.V.) by quantitative TLC[2].

The above data established that the solvent used for spotting a standard solution affected precision. Next, it was of interest to determine if the solvent used for reconstitution of residues from extracts of biological samples affected reproducibility of an assay in the same way. Replicate 1 ml samples (n=9) of plasma spiked with II (500 ng) were extracted with chloroform (6 ml) and centrifuged. The chloroform was divided into two 2 ml portions which were evaporated to dryness. One portion was redissolved with chloroform while the other portion was redissolved with methanol-chloroform (15:85) and both were spotted and quantitated on the same silica gel TLC plate. The C.V. for the samples of II applied in a methanol-chloroform solution was ±1.8% while that for the chloroform solution was ±4.2%. Compound IV was treated in an analogous manner after extracting it from acidified plasma (1 ml) with 1,2-dichloroethane (6 ml). A ±13.8% C.V. in mean peak area was obtained with the reconstituted chloroform samples, whereas the C.V. was ±4.1% with the methanol mixture.

When recovery experiments were performed with compounds II and IV over a range of concentrations (20-4000 ng/ml) using a methanol-chloroform (15:85) solution for reconstitution instead of chloroform, the precision was improved substantially. The deviation from the regression of added vs recovered drug, when expressed as the percentage variance about the mid-point ($\bar{X}$,$\bar{Y}$) of the regression[3], decreased from 15% with pure chloroform for II and IV to 4% and 2.8%, respectively, with the methanol mixture.

Standard solutions (n=10) of compounds II, III, and IV dissolved in chloroform were also analyzed by HPLC and quantitated by peak-height measurement. C.V.'s were 1.9%, 4.7%, and 1.1%, respectively (Table II). This HPLC experiment indicated that the solvents investigated did not affect the quantity of compound that was applied to the TLC plate, but suggested that dispersion of the compound on the plate or adsorption to the silica gel in the presence of the developing solvent was important.

To determine which of these two factors affected reproducibility, II and IV were spotted with either methanol-chloroform (15:85) or chloroform onto plates which were either untreated or were pre-developed (3 cm) in a polar solvent to deactivate the silica gel prior to spotting. Plates spotted with drug were scanned with a spectrodensitometer prior to and after development. Results indicated a substantial reduction in the C.V. for compounds in chloroform solutions when the pre-developed silica gel plates were used (Table III). Compound II had a C.V. of ±23% with untreated plates and ±3.7% for predeveloped plates. The reproducibilities of the measurement of IV when spotted in a methanol-chloroform (15:85) solution on pre-developed plates as compared to untreated plates were nearly equivalent.

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TABLE II

REPRODUCIBILITY OF MEASUREMENT FOR COMPOUNDS II, III & IV BY HPLC

HPLC DATA

Compound No.	Injected Amount	Injected Volume	n	Coefficient of Variation
II	250 ng	50 µl	10	±1.9%[a]
III	160 ng	20 µl	10	±1.1%
IV	50 ng	50 µl	10	±4.7%

[a] The same result was achieved when the same syringe was used without intermediate cleanings.

TABLE III

EFFECT ON PRECISION OF PLATE PRE-DEVELOPMENT IN A POLAR SOLVENT

Compound	Solvent[d]	Coefficient of Variation			
		Regular Plate		Pre-developed Plate[b]	
IV	B	±4.9%	(12.8%)[a]	±4.1%	n = 27
	A	±24.5%	(17%)	±5.5%	n = 25
II	B		(±4.2%)	-	-
	A		(±23%)	±3.9%[c]	n = 14

(a) ( ) indicates data taken from Table I.

(b) The pre-development solvent for IV was $MeOH:CHCl_3$ (1:1), while II was pre-developed in $H_2O:IPA:MeOH:CHCl_3$ (2:10:20:60).

(c) The polarity of the developing solvent system for II was reduced to obtain an Rf of 0.5.

(d) Application solvents: A, chloroform; B, methanol-chloroform (15:85).

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Deactivation of the silica gel at the origin during the spotting process was further implicated when replicates of compound IV gave a C.V. of ±17% when spotted directly in chloroform, yet showed only a ±3.4% C.V. when this same chloroform standard was first shaken with water prior to spotting.

DISCUSSION

In reviewing the sample preparation schemes for various quantitative TLC methods that have been described in the literature, it is clear that different solvents have been used for application of the samples to TLC plates. The reason for choosing a particular solvent is generally not given; however, some investigators apparently try to use the least polar solvent that will dissolve the residue. The data presented here suggests that solvents used for sample application can have a dramatic effect on the precision of the method. When the effects of solvent on precision were first observed in our laboratory, several factors which might be responsible were considered: (1) evaporation of the solvent on the needle tips during the spotting process, (2) compound solubility, (3) salt or complex formation, (4) glass adsorption, (5) degree of dispersion of the solute at the origin during application and (6) relative affinity of the solute for the silica gel. Evaporation of the solvent on the

tips of the needles could not be a contributing factor because that variable is expected to be solvent dependent rather than compound dependent. For example, phenacetin had a C.V. of 1.2% while aspirin had a C.V. of ±28% when both compounds were applied onto the plate in chloroform. Drug solubility was excluded as a source of error since the concentrations of the solutions prepared were kept well below their solubility limits. Solubility and salt formation are important in the extraction and concentration phase of sample preparation. Proteins, lipids, and various inorganic ions are concurrently extracted with the drug and upon concentration may form complexes and salts with the drug. This process may alter the solubility of the drug in the solvent used for reconstitution and may also affect the diffusion of the drug through the layer of silica gel. When the sample is spotted, the deposit of extraneous material at the origin may also effect migration of the developing solvent up the plate. This factor should affect reproducibility, but in the current situation since standard solutions with pure solvents were used, this would not explain the poor precision.

Adsorption to glass is a property of some molecules that can cause problems with reproducibility for some analytical methods. This problem can be excluded as a factor in the current work since HPLC analysis of chloroform solutions of II, III, and IV had acceptable reproducibilities.

After dismissing factors not contributing significantly to the effect of solvent on reproducibility, attention was focused on how solvent affected the interaction between the solute and the plate surface. The size of the spots at the origin would be expected to vary as the methanol-chloroform mixture might diffuse the compound more than chloroform alone. However, visualization of the spots at the origin with UV light (F-254 silica gel plates) indicated that there were no observable differences between the size nor distribution of the compounds when spotted with the two solvents. One is left with the probable explanation that methanol deactivates the silica gel and lessens the hydrogen bonding between the silica gel and the more polar solutes. The amount of a compound that will be retarded at the origin will depend upon the quantity of the solute, the nature of coextractives in the sample and the activity of the silica gel. Apparently, the material that adheres to the origin is gradually displaced and moved up the plate during the development process, since scans of the origin after development of the plate show no compound remaining. When silica gel plates are partially deactivated by pre-development (3 cm) in a polar solvent system (see Table III) prior to spotting a compound (even in a chloroform solution), acceptable reproducibilities are obtained.

It is apparent from these studies that the solvent used for spotting a compound on a TLC plate can affect the reproducibility of an assay. This

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conclusion is supported by the recent report of Scott and Kucera[4] who studied solute-solvent interactions on the surface of silica gel. They concluded that silica gel in contact with solvents adsorbs solvent molecules to form bilayers. Polar or hydrogen-binding solvents can displace less polar solvents already bound, while solutes (in low concentrations) displace the outer layers of solvent molecules but do not interact directly with the surface of the silica gel itself. For solutes that have a polarity similar to the solvent, competition with the primary layer can occur and solutes may interact directly with the silica gel surface. The differences between the interaction of polar or non-polar solutes with silica gel is a factor which previously has not been given much attention, but must be considered when developing a quantitative TLC procedure.

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REFERENCES

1. J.G. Kirchner, J. Chromatog., 82, (1973) 31.
2. W. Hezel, American Lab., May (1978) 91.
3. G.W. Snedecor and W.G. Cochran, Statistical Methods, Sixth Edition, Ch. 6, Iowa State University Press, Ames, Iowa 1967.
4. R.P. Scott and P. Kucera, J. Chromatog., 171, (1979) 37.