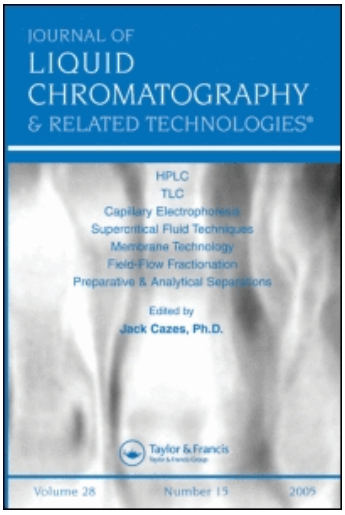

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EVALUATION OF SYSTEM ERRORS IN TLC-DENSITOMETRY WHEN USING THE STREAKING TECHNIQUE

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ABSTRACT

The errors that contribute to the total variance of the automated streaking technique are examined. These results are compared with computer optimized and zig-zag scanning approaches. The error due to inhomogeneous sample distribution is shown to be the most important.

INTRODUCTION

Quantitative Thin Layer Chromatography (TLC) is a legitimate supplement or alternative to High Performance Liquid Chromatography (HPLC) (1). This is especially true with pharmaceutical analyses where the sample matrix is often complex and cannot be injected directly onto expensive columns.

Within the past few years, the art of TLC densitometry has made significant advances. Coupled with high performance TLC (2, 3, 4) excellent performance capabilities are now attainable.

One of the chief limitations of densitometry is the relatively narrow dynamic range of sample mass (5). It is well known that non-uniform

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distribution of the sample within the layer causes nonlinear results. To some extent, this difficulty can be overcome by one of three approaches: streaking the sample and scanning the center of the streak; scanning with a small beam in a zig-zag manner; or controlling the positioning of the beam by computer. Each of these approaches has its advantages. For the streaking technique, there is less variation in sample distribution ; zig-zag scanning can overcome differences in spot shape; and the computer controlled system can optimize scanning position.

This paper evaluates the factors that contribute to the overall system error when using an automated streaking device, and compares them with what is attainable by the computer controlled and zig-zag scanning approaches.

EXPERIMENTAL

Sample solutions were prepared in methanol at the following levels: Caffeine (J. T. Baker, USA), 0.30 mg/ml and 2.50 mg/ml; theophylline (J. T. Baker, USA), 0.28 mg/ml; Sunset Yellow (Nederlandsek Kleurstafindustrie, Holland), E110, 1.0 mg/ml; Patent Blue, E131, 1.0 mg/ml. All samples were applied with a Camag Linomat III. Streaks were 10 mm wide and applied at a rate of 50 mm/µl and 5 sec/µl. Conventional 20 x 20 cm TLC plates were purchased from Merck (Alltech Associates, USA) coated with Silica Gel 60F. The 10 x 10 cm high performance plates were purchased from Merck and also coated with Silica Gel 60F. High performance plates were developed three centimeters in a 4" x 4" x 3" chamber, while the conventional plates were developed six cm in a Camag Twin Trough Chamber. Caffeine linearity, and sample volume studies were accomplished on conventional Merck plates and developed a distance of 10 cm. The two dye samples were developed with isopropyl alcohol:ammonia, 75:25, v/v, without preequilibration; while caffeine and theophylline samples were developed with chloroform:methanol,

90:10, v/v, after 15 minutes of preequilibration in a paper-lined TLC chamber. Developed streaks were scanned using a Schoeffel SD3000 (Schoeffel Instrument Corp., Westwood, NJ, USA) densitometer with a 5 mm beam. The Sunset Yellow streaks were scanned at 500 nm, the Patent Blue at 650 nm, and the caffeine and theophylline at 280 nm.

RESULTS AND DISCUSSION

Contributions to the total variance of any TLC process has been described by Ebel (6). The system variance, σ^2 is the sum of the square of the sample application error, σ_v; the chromatography error, σ_c; the measurement error, σ_m; and the positioning error, σ_p.

$$\sigma^2 = \sigma_c^2 + \sigma_p^2 + \sigma_v^2 + \sigma_m^2$$

The measurement error can be estimated by scanning the same spot repeatedly without recentering the beam before each scan; whereas, the positioning error can be obtained by repetative scanning of the same spot after reoptimizing the beam. The sample application and chromatography errors are not as easily established. Determination of the sample application error has been done by assuming that under highly efficient conditions, the chromatographic error is zero (7). Because the chromatography parameter is usually the largest and most difficult to control, this assumption is tenuous.

The chromatography error is related to the physical and chemical aspects of each particular system. However, as far as the densitometric process is concerned, the two most important manifestations of chromatographic variance are the shape of the developed spots and the distribution of the sample within the plate matrix. Because of the open bed aspect of TLC, lateral sample diffusion is often a significant factor in causing spot shape variation and different shaped spots can also yield different responses (8). Also, because of the previously mentioned narrow sample mass dynamic range, variations in

sample distribution can cause significant error (5). Accordingly, the chromatographic variance, σ_c^2, is more accurately described by a spot shape variance, σ_s^2, and a sample distribution variance, σ_d^2. Thus, the total variance becomes:

$$\sigma^2 = \sigma_m^2 + \sigma_p^2 + \sigma_v^2 + \sigma_s^2 + \sigma_d^2$$

In comparing different densitometric modes, each of these errors must be considered.

With computer controlled optimization, the beam positioning error is negligible (9).

$$\sigma^2_{computer} = \sigma_m^2 + \sigma_v^2 + \sigma_d^2 + \sigma_s^2$$

Small beam, two dimentional scanning (zig-zag) devices effectively eliminate variations due to both position and spot shape (8). In addition,

$$\sigma^2_{zig\text{-}zag} = \sigma_m^2 + \sigma_v^2 + \sigma_d^2$$

to some extent the problem of non-uniform sample distribution is not as significant when using a small beam scanned in two dimensions. Whereas, the detector response for a long narrow beam gives an average reading which may or may not reflect the amount of sample present, a smaller beam is not as adversely effected. However, due to the relatively short dynamic range inherent with densitometry, even small beams may not overcome this problem.

The ability of the automated streaking device to minimize the sample volume, beam position, and spot shape errors, was demonstrated by applying 16 µl of a similar 0.25 mg/ml caffeine in methanol solution and 20 µl of a similar 0.20 mg/ml caffeine solution. Since the second solution is exactly 80% of the first one with respect to concentration, equal amounts of caffeine can be applied only if the automated streaking device is able to deliver accurate volumes of sample. Table 1 shows that four streaks of each solution

TABLE I

Densitometric Responses for Different Volume of Solution

	4 µg Caffeine		2 µg Caffeine	
	20 µl	16 µl	20 µl	8µl
x	59.2 x 10^4 area	59.5 x 10^3 area	40.6 x 10^4 area	39.8
SD	2.1	1.2	2.0	1.1
RSD	3.5%	2.0%	4.9%	2.8%

gave equivalent densitometric results according to the null hypothesis when scanned at 280 nm, where the probability of the difference between the two means being greater than zero is less than 60%. Also, 8 µl of 0.25 mg/ml caffeine solution was compared with 20 µl of 0.1 mg/ml caffeine solution. Similarly, the amount of caffeine delivered to the plate by both solutions was the same when scanned, where the probability of the difference between the two means being greater than zero is only 75%.

The automated streaking device is also capable of delivering linear amounts of caffeine solution. As seen in Table 2, the linearity is the same by streaking different concentrations (0.05 to 0.25 mg/ml) of analyte at fixed volume (20 µl), as that obtained by applying changing volumes (4-20 µl) of a fixed concentration (0.25 mg/ml) solution.

If a sample application error were significant, it would be a function of the total volume of sample applied, since the chromatographic measurement and positioning errors are the same in both systems. Clearly, there is no apparent difference in how the same amount of a sample is applied, when the center of the streak is scanned. This conclusion is reenforced by the results obtained when streaking equivalent linear amounts of caffeine from a fixed volume while varying sample concentration versus varying volumes of a fixed concentration solution (Table 2).

Additionally, because the beam position was not optimized during these measurements, the σ_p error is eliminated.

TABLE 2

Linear Regression Analysis of Densitometric Scans of Equivalent Amounts of Caffeine Streaked from Different Volumes of Solutions

	Volume Change[1]	Concentration Change[2]
x mean	2.5%	2.5%
y mean	43.2×10^4 area units	42.5×10^4 area units
correlation coefficient	0.993	0.974
relative y-intercept	20.7%	14.8%
intercept	9.0×10^4 area units	6.3×10^4 area units
slope	1.4×10^5 area units/μg	1.4×10^5 area units/μg

[1]Applied by streaking 4, 8, 12, 16, and 20 μl of 0.25 mg/ml caffeine solution.

[2]Applied by streaking 20 μl of 0.05, 0.1, 0.15, 0.20, and 0.25 mg/ml caffeine solution.

The major errors from automated streaking are related to the chromatographic factors, σ_s and σ_d. With streaking, as long as the solvent front travels uniformly, the shape of the center of the streak will not contribute significantly to the overall variance. Further, shape distortions are much easier to observe with streaks than with spots. Streaks that are distorted or not perpendicular to the flow of solvent should be quantitated. When this is done, only the sample distribution error contributes measurably to chromatographic variance. Thus,

$$\sigma^2_{streaking} = \sigma_m^2 + \sigma_d^2.$$

Precision data for sight replicate streaks of four different compounds are listed in Table 3. The two dyes are large ionic compounds, with low diffusion coefficients, and are therefore less subject to sample inhomogeneity, *i.e.*, σ_d^2 is small. On the other hand, theophylline and caffeine are more prone to diffusion and this is reflected in slightly larger RSD values.

TABLE 3

Standard Deviation of Samples Applied by the Streaking Technique

Compound	RSD, % (peak height)
1. Patent Blue, E-131	
HPTLC, σ	1.9
TLC, σ	1.6
2. Sunset Yellow, E-110	
HPTLC, σ	1.6
TLC, σ	1.6
3. Theophylline	
HPTLC, σ	2.5
TLC, σ	2.3
4. Caffeine	
HPTLC, σ	2.6
TLC, σ	1.1

In actual practice, contributions to the total variance for all three modes of analysis may be simplified. When samples are applied manually with 0.5 µl microcapillaries, the relative application error is less than 1%. Also, the electronics in densitometers keeps the measurement error around 0.2-0.6% (9). This leaves to the spot shape, and sample distribution errors as the main sources of imprecision. As described above, the variance from automated streaking and zig-zag scanning will mainly depend upon the sample distribution error; while the variance from computer controlled optimization will be related to both spot shape and sample distribution. These two factors remain the Achilles heel of densitometry. However, when any of the above densitometric approaches is coupled with a modern high performance TLC system, percent variations of 1-3% can be expected.

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