Quantitative HPTLC

by

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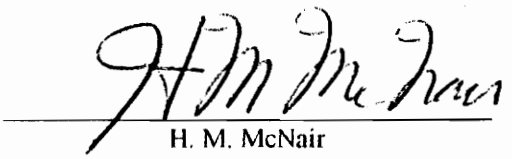
Thesis submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Masters of Science

in

Chemistry

Approved: 
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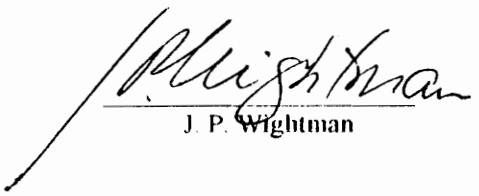
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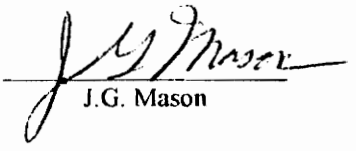
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December, 1995

Blacksburg, Virginia

QUANTITATIVE HPTLC

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(ABSTRACT)

Advances in thin layer chromatography (TLC), including smaller more uniform particles, use of a scanning spectrophotometer (densitometer) and sample application devices, led to the development of the High Performance Thin Layer Chromatography (HPTLC) technique. HPTLC allows quantitative as well as qualitative results of much smaller amounts, in some cases down to the picogram level. With these advancements, the limiting factor in detection of smaller concentrations has become the plate itself, and more specifically the preparation of the absorbent and binder and the layering process.

This research evaluated HPTLC plates from several manufacturers for significant differences between manufacturers and between plates of each manufacturer. Several concentrations of three drugs of abuse were applied, developed and quantitated. Both Rf and peak area were statistically evaluated to look for any effect of manufacturer, specific plate for that manufacturer, specific drug, concentration and/or cross nested effects.

Significant difference were found between manufacturer for both Rf and peak area, with E. Merck and Baker plates having the best overall results. All manufacturers were found to have some plates with obvious visual surface defects that were not suitable for use. The major source of variation for all manufacturers was the plate to plate variation rather than track to track deviations on any given plate.

ACKNOWLEDGMENTS

This student would like to thank the Statistics Department of Virginia Polytechnique Institute & State University for all their help with the programming and processing of the data. Thanks also to the Chemistry Department for the use of the facilities and the truths uncovered within the domain of chemistry. Special thanks go to this student's committee for their patience and understanding during the research and writing process. Special thanks go the this student's advisor, Harold M. McNair, Ph.D., who patiently taught this student and gave the needed advise to achieve the final product, this thesis.

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INTRODUCTION

In 1904, chromatography was invented by Michael Tswett, who performed a separation of chlorophyll by passing it through a calcium carbonate column with petroleum ether as the mobile phase. He achieved distinct bands of yellow, purple and green[1]. This experimental observation led to his naming the technique "chromatography" or color writing. This beginning was largely ignored until the 1930's when α and β carotene were separated by Kuhn, Leaderer and Winterstein[2]. This preparative scale experiment verified Tswett's prediction that the "components of a mixture of pigment are separated in a definite order and can then be determined qualitatively and quantitatively"[3].

This Liquid Solid Chromatography (LSC) mode continued developing throughout the 1930's. Starting in 1930, thin layer chromatography (TLC) was established with the introduction of "chromatostrips"[4]. These were narrow pieces of flat glass with the stationary phase being a layer of aluminum oxide on one side. By 1950, little research using this technique had been published, since the results obtained could not be reproduced between laboratories[5]. This changed in 1958[6] when Egon Stahl published a classical paper on TLC. In his paper, he carefully described the entire technique from the preparation of the stationary phase, silica gel with binder, to the layering process. This standardized TLC technique allowed research laboratories to reproduce one another's work. Shortly thereafter the commercial market was selling the preparations and devices for the manufacture of TLC plates. Research in TLC expanded rapidly.

The 1960's brought with them the development of High Performance Liquid Chromatography (HPLC), a liquid-solid chromatographic technique utilizing high pressure pumps to assist solvent flow. In the United States, research was redirected to HPLC and away from TLC, unlike in Europe where TLC remained prominent. The major research thrust in the US was in the development of a variety of high

quality stationary phases for HPLC. Tests revealed that peak resolution was dependent upon particle diameter of the stationary phase, i.e. the smaller particles showed higher chromatographic resolution. Problems arose when, using the smaller particles, the pressure drop across the normal length columns was so great that traditional pumps could not be used. As a result the columns were shortened. These shorter columns with smaller particles had a second advantage -- faster separation times.

When the smaller particles were introduced to the TLC technique, similar results were obtained with a slightly thinner absorbent layer (200μm instead of 250μm) and smaller particles (7μm instead of a range of 5 to 40 μm[7]), the separation time was significantly shortened and resolution was improved. The problem, however, was that sample capacity decreased as layer thickness decreased. This restricted the visualization technique used to quantitate and qualitate the individual components of a mixture. The development of a uv/vis scanning spectrophotometer was essential for this improved technique.

The first scanning spectrophotometer (densitometer) was introduced in the 1970's by CAMAG, of Muttenz Switzerland, and became a standard detector for TLC making human visualization of the plate no longer necessary. The densitometer output signal was the measured difference between the plate background (i.e. the response of the silica gel) and the sample spot. This differential signal is sent to a recorder yielding a chromatogram similar to a HPLC chromatogram. Use of a densitometer allows detection of lower minimum detectable quantities, and, in some cases, picogram levels[8] can be obtained, compared to 1 - 10μg[9] achievable by visual inspection. This allowed TLC to become a quantitative technique

At the same time, CAMAG also introduced sample application devices. The sample applicators were designed to deliver reproducible sample spots at reproducible positions along the edge of the plate further reducing errors in the quantifying of results. The combined use of these applicators, the densitometer, and the 7μm particle thin layer plates became what is now known as High Performance

Thin Layer Chromatography. The limiting factor in detection of smaller concentrations is now the plate itself, and more specifically the preparation of the absorbent and binder and the layering process.

The first set of questions this research posed were "Are there significant differences among different manufacturer's plates?" and "Do the differences between manufacturers make a difference for the user on individual scans?" The first question was explored by running samples on a collection of plates from different manufacturers and analyzing the results. The second question was explored by determining an "unknown" concentration on each manufacturer's plate with the aid of a calibration curve. The calibration curve, the external standard, negated the linearity differences of the plates within a given manufacturer.

Upon closer look at the plates of individual manufacturers, the second set of questions posed were "Are the plates uniform throughout a given batch?" and "Is the layer of the individual plate uniform?" Experiments were designed to measure non-uniformity between the plates of a given manufacturer. This difference is known to be present and accepted by industry. Individual plate non-uniformity was monitored by developing several sample tracks on a given manufacturer's plate. This procedure served to test the precision of the spotting device as well. The reproducibility of the scanning device was tested by densitometrically scanning the sample tracks twice for all manufacturers' plates and comparing the results. A single manufacturer's plate was also scanned multiple times to determine instrumental precision.

The third set of questions posed was "Are the results dependent on the specific compound and concentration investigated?" and "Are the results dependent on how soon after development the plates are scanned (in days)?" This study used three drugs of abuse: cocaine, codeine and methadone. The Rf (the migration distance of the sample divided by the migration distance of the solvent) results were examined and compared to quantitate variance between manufacturers. The peak area dependence on concentration

(i. e. linearity) was analyzed by further examining the calibration curve data. A set of plates were analyzed on day one and then three days later, results were statistically compared.

EXPERIMENTAL

APPARATUS

The spot application was accomplished by using the CAMAG Linomat IV or the CAMAG Nanomat II (CAMAG, Muttenz, Switzerland). The plates were developed in a twin trough chamber and then dried with a cool air blower. After development, the plates were densitometrically scanned using the CAMAG Scanner II with a SP-4270 Spectra Physics integrator (Sunnyvale, California).

REAGENTS AND MATERIALS

The drug standards (cocaine, codeine and methadone - Figure 1) were purchased as hydrochloride salts in methanol solutions (1 g/ml) from Sigma Chemical Company (St. Louis, MO). The developing solvents (ethyl acetate, methanol, and aqueous ammonia) were HPLC grade from EM Science (Gibbs Town, NJ). The 10 X 10cm plates were silica gel high performance thin layer plates with a 200μm thickness and a fluorescent indicator at λ=254nm; the exception was Analtech's plates which had a 150μm layer thickness. All available HPTLC plates were ordered from all existing manufacturers known to this researcher. Only two manufacturers were not evaluated for reasons explained in Appendix 1. The manufacturers' plates evaluated were Alltech Associates, Inc. (Applied Science Labs, Deerfield, IL) with its High Speed TLC plates; Analtech (Newark, DE) with HPTLC-HLF plates; Baker Chemical Company (Phillipsburg, NJ) with Si-HPF plates; E. Merck (Darmstadt, Germany) with Silica Gel 60-F254 plates; and Whatman (Clifton, NJ) with HP-KF plates. All plates were purchased in the spring of 1986.

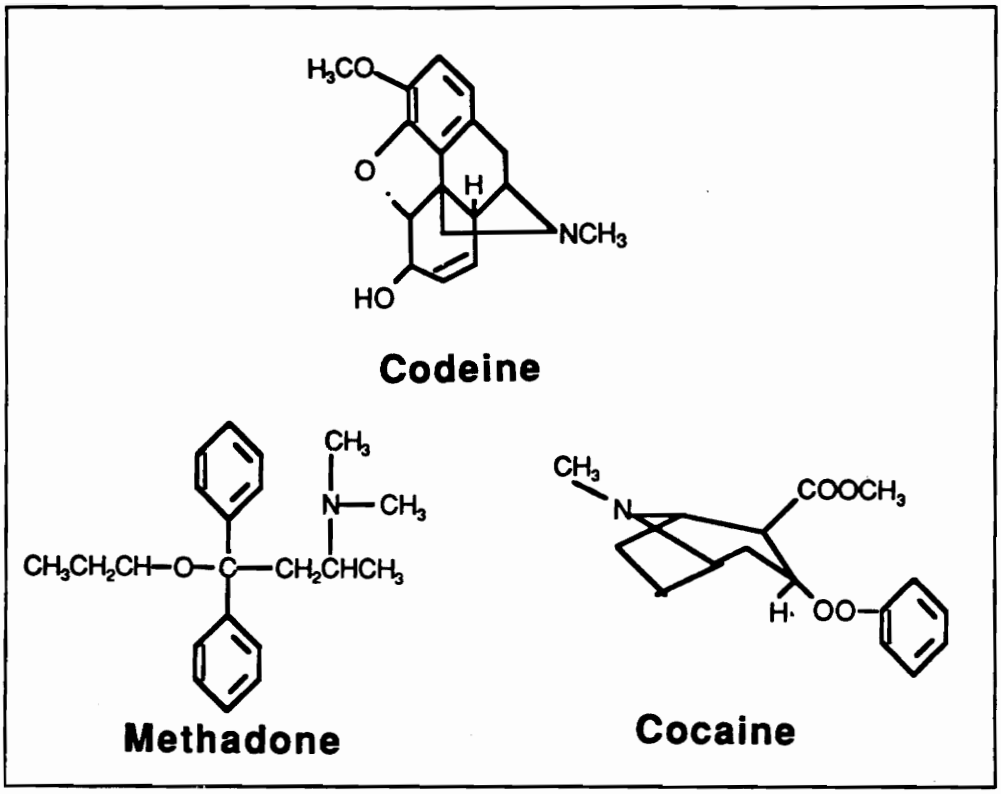


Figure 1 - Chemical Structures of Drugs Tested

ANALYTICAL PROCEDURE

The analytical HPTLC plates were prewashed prior to sample application. This was done by a batch washing method[10] of dipping the plate in isopropanol and then drying the plate with a cool air blower. This process was repeated 4 times, with each dipping into fresh isopropanol. Heating the plate in a 105°C oven for one hour activated the plates. Plates were transferred to a desiccator for cooling and storage.

The purchased drug standards (1 mg/ml) were combined and diluted 1:10 in methanol yielding a solution concentration of 0.1 mg/ml. The combined standard solution was then applied to the plates.

TEST 1 - General Manufacturer Plate Comparison

Five microliters of the combined drug standard was applied in bands to the four plates of each manufacturer using CAMAG Linomat IV. There were three 6mm x 1mm bands placed 7mm from the bottom edge of the plate and 35mm from the left side edge with a 4mm space between each band. Preconditioning of the plates in the solvent system's vapor was for five minutes, ethyl acetate/methanol/ammonia(aq) (17:2:1)[11]. Development of the plates was allowed to progress until solvent migration was 50mm from the bottom edge. Plates were dried immediately after development with a cold air blower.

Each plate was scanned spectrophotometrically with the CAMAG Scanner II using the absorbance mode, a slit width of 3mm positioned in the center of each sample band, the mercury lamp with its setting on λ=254nm and scanning speed of 1mm/sec. Each track was scanned twice. Resultant peak areas were integrated using the SP-4270 integrator and reported using the CAMAG TLC 6000 report format program.

TEST 2 - Signal to Noise--E. Merck & Baker Chemical Co.

Based on the results of Test 1, the two manufacturers with the best reproducibility were chosen to perform the signal to noise (S/N) ratio evaluation. (Signal to noise evaluation was not performed on all manufacturers due to scanner usage time constraints.) One prewashed and activated plate from each manufacturer was chosen. Plates were then spotted with 1µl of the combined drug standard 7mm from the bottom edge in the center portion of the plate with the CAMAG Nanomat II. The plate was preconditioned in the solvent system vapor, developed and dried as described in Test 1. The plates were scanned spectrophotometrically at the conditions listed in Test 1 with the exception of a deuterium lamp as the incident light source. A wavelength analysis was performed obtain the maximum compound response. To do this the wavelength was incremented by 10nm beginning at 220nm and going to 300nm. The wavelength 230nm gave the largest response for both manufacturers' plates. The densitometer's

sensitivity setting needed to be raised from 145 to 160 on the 250 scale in order to magnify the signal and the noise to full scale in order for a manual height measurements of both to be made. The integrator's attenuation was increased to 32 from 64 and the chart speed was increased to 8 cm/min from 4 cm/min. Signal to noise calculation was the division of the peak height of each drug by the greatest noise height difference.

TEST 3 - Single Manufacturer Plate Comparison

Based on the signal to noise results from Test 2, E. Merck Plates were used to evaluated batch deviations within a single manufacturer. Ten prewashed and activated E. Merck plates were spotted with the combined drug standard (1mg/ml of each drug) using the CAMAG Linomat IV. Each plate had 6 tracks of combined drug standard applied in bands 6mm x 1mm and 7mm from the bottom edge, three tracks with 1µl of the standard (100ng) and three tracks with 3µl of the standard (300ng). The plates were preconditioned, developed and dried as described in Test 1. The plates were spectrophotometrically scanned in twice using the condition in Test 1 with the exception of a deuterium lamp incident light source with λ=230nm. The wavelength of 230nm gave the highest intensity absorbance of all three drugs on the E. Merck plates. This test was repeated 3 days later.

TEST 4 - Determination & Comparison For Linearity Range Calibration Curves

Two prewashed and activated plates from each of the five manufacturers were selected. The plates were spotted using the CAMAG Nanomat II. Six known volumes of the combined drug standard were applied 7mm up from the bottom edge of the plates: 0.5µl, 1.0µl, 1.5µl, 2.0µl, 2.5µl, 3.0µl. The plates were preconditioned, developed and dried as in Test 1. The plates were scanned spectrophotometrically with the same conditions as in Test 3. The manufacturers' plate peak area counts of each drug were plotted against the concentration, providing a calibration curve. An "unknown"

concentration was selected, 150ng (1.5μl volume), and determined using the calibration curve. This determined concentration was compared to its known concentration.

STATISTICS

The statistics were done by the Virginia Polytechnique Institute and State University's Statistics Department. The Statistic Department used the SAS software package (SAS, Inc., Cary, North Carolina) and specifically used its General Linear Model Procedure, F-test and T-test. For more details of each test refer to the references cited.[12,13,14,15]

STATISTICS - TEST 1

A linear model (Equation 1) was formulated by the Statistics Department which contains both fixed and random effects to analyze manufacturer's variances. The fixed effects are the plate manufacturer and the specific drug. Variable effects are Rf (the distance the sample migrates divided by the distance the solvent front migrates) and area between scans, tracks, plates, manufacturers and drugs. The fixed effects of plate manufacturer and the specific drug are cross-nested within each other, illustrating that the effects are co-dependent upon each other. Interactions are also estimated from this model. Random effects are based on the uncontrolled variations within the fixed effects, such as the different plates, tracks or scans. Random effects are nested within a fixed effect, indicating that for the fixed effect of the known manufacturer, an unknown plate from that manufacturer is chosen. Random effects can be estimated from the variance components which are given to the fixed effect. The results were the random effects deviation or variance components.

Equation 1

$$y_{ijklm} = \mu + q_i + p_{ij} + s_{ijk} + d_l + qd_{il} + pd_{ijl} + sd_{ijkl} + \varepsilon_{ijklm}$$

where:

μ	=	the overall mean
q_i	=	the i^{th} manufacturer effect (i = 1, 2, 3, 4, 5)
p_{ij}	=	the j^{th} plate effect within the i^{th} manufacturer (j = 1, 2, 3, 4)
s_{ijk}	=	the k^{th} track effect within the j^{th} plate of the i^{th} manufacturer (k = 1, 2, 3)
d_l	=	the l^{th} drug effect (l = 1, 2, 3)
qd_{il}	=	the interaction effect of the i^{th} manufacturer and the l^{th} drug
pd_{ijl}	=	the interaction effect of the j^{th} plate of the i^{th} manufacturer and the l^{th} drug
sd_{ijk}	=	the interaction effect of the k^{th} track on the j^{th} plate of the i^{th} manufacturer and the l^{th} drug
ε_{ijklm}	=	the m^{th} scan effect of the k^{th} track on the j^{th} plate of the i^{th} manufacturer (m = 1, 2)
y_{ijklm}	=	response variable (either Rf or area)

STATISTICS - TEST 2

These variance components provided the needed information for the differences within a given manufacturer, but did not compare manufacturers. This comparison was accomplished by performing the F-test[16] on the hypothesis

$$\sigma^2_{plate} = 0$$

where σ^2 is the variance component of the plate for different manufacturers. Since the observed result for each manufacturer was not equal to zero,

$$\sigma^2_{plate} \neq 0,$$

a Satlerthwaite-type[17] F-test of the synthesized mean squares was used, thereby allowing a pairwise comparison of the manufacturers mean squares for both Rf and area.

STATISTICS - TEST 3

To statistically test for an interaction or dependence between the drug standards and the manufacturers, the hypothesis

$$H_o = \mu_{ANALTECH} = \mu_{ALLTECH} = \mu_{BAKER} = \mu_{E.\ MERCK} = \mu_{WHATMAN}$$

was performed on each manufacturer using a F-test. A significant difference was observed between manufacturers. Therefore, a pairwise T-test using least square means[18] was also performed. The T-test, using least square means, was utilized since the data set from each manufacturer was not equal, i.e. there were not the same number of data points for each manufacturer for a given drug, thus giving an unbalanced data set.

Next, this hypothesis was tested for each drug by a F-test. Consequently, a pairwise T-test using least square means was completed and a significant interaction was observed. A second statistical procedure was made on another linear model (Equation 2), containing both fixed and random effects. The fixed effects include the type of drug, day of test and the concentration. The random effects encompass the plates, tracks, and scans.

The F-test and Duncan's T-test[19] were initiated in order to compare the data base on the separate concentrations using another linear model (Equation 3). Duncan's T-test was used because the data set was balanced, i.e. there were the same number of data points in each fixed or random effect group.

STATISTICS - TEST 4

The final test on this set of data was based on the linear model in Equation 4. This data is significant for each test variable for each drug, and was followed by Duncan's T-test to give the overall mean difference of each variable for the comparison of drugs.

Equation 2

$$y_{ijklmn} = \mu + b_i + p_{ij} + d_k + c_l + (dc)_{kl} + (bd)_{ik} + (bc)_{il} + (bdc)_{ikl} + (pd)_{ijk} + (pc)_{ijl} + (pdc)_{ijkl} + s_{ijklm} + \varepsilon_{ijklmn}$$

where:

μ	=	the overall mean
b_i	=	the i^{th} day effect (i = 1,2)
p_{ij}	=	the j^{th} plate effect on the i^{th}day (j = 1, 2, . . ., 10)
d_k	=	the k^{th} drug effect (k = 1, 2, 3)
c_l	=	the l^{th} concentration effect
$(dc)_{kl}$	=	the interaction effect between the k^{th} drug and the l^{th} concentration
$(bd)_{ik}$	=	the interaction effect between the i^{th} day and the k^{th} drug
$(bc)_{il}$	=	the interaction effect between the i^{th} day and the l^{th} concentration
$(bdc)_{ikl}$	=	the interaction effect between the i^{th} day, the k^{th} drug & the l^{th} concentration
$(pd)_{ijk}$	=	the interaction effect of the j^{th} plate on the i^{th} day and the k^{th} drug
$(pc)_{ijl}$	=	the interaction effect of the j^{th} plate on the i^{th} day and the l^{th} concentration
$(pdc)_{ijkl}$	=	the interaction effect of the j^{th} plate on the i^{th} day,the k^{th} drug & the l^{th} concentration
s_{ijklm}	=	the m^{th} track effect within the j^{th} plate on the i^{th} day with the l^{th} concentration of the k^{th} drug (m = 1, 2, 3)
ε_{ijklmn}	=	the n^{th} scan effect of the m^{th} track on the j^{th} plate on i^{th} day with the l^{th} concentration of the k^{th} drug (n = 1, 2)
y_{ijklmn}	=	response variable (either Rf or area)

Equation 3

$$y_{ijklm} = \mu + b_i + p_{ij} + d_k + (bd)_{ik} + (pd)_{ijk} + s_{ijkl} + \varepsilon_{ijklm}$$

where:

μ	=	the overall mean
b_i	=	the i^{th} day effect (i = 1, 2)
p_{ij}	=	the j^{th} plate on the i^{th} day effect (j = 1, 2, . . ., 10)
d_k	=	the k^{th} drug effect (k = 1, 2, 3)
$(bd)_{ik}$	=	the interaction effect of the i^{th} day and the k^{th} drug
$(pd)_{ijk}$	=	the interaction effect of the j^{th} plate on the i^{th} day and the k^{th} drug
s_{ijkl}	=	the l^{th} track effect within the j^{th} plate on the i^{th} day within the k^{th} drug
ε_{ijklm}	=	the m^{th} scan effect within the l^{th} track on the j^{th} plate on the i^{th} day within the k^{th} drug
y_{ijklm}	=	the response variable (either Rf or area)

Equation 4

$$y_{ijlmn} = \mu + b_i + p_{ij} + c_l + (bc)_{il} + (pc)_{ijl} + s_{ijlm} + \varepsilon_{ijlmn}$$

where:

μ	=	the overall mean
b_i	=	the i^{th} day effect (i = 1, 2)
p_{ij}	=	the j^{th} plate effect on the i^{th} day (1, 2, . . ., 10)
c_l	=	the l^{th} concentration effect
$(bc)_{il}$	=	the interaction effect of the i^{th} day and the l^{th} concentration
$(pc)_{ijl}$	=	the interaction effect of the j^{th} plate on the i^{th} day and the l^{th} concentration
s_{ijlm}	=	the m^{th} track effect within the j^{th} plate on the i^{th} day with the l^{th} concentration
ε_{ijlmn}	=	the n^{th} scan effect within the m^{th} track on the j^{th} plate on the i^{th} day with the l^{th} concentration
y_{ijlmn}	=	the response variable (either Rf or area)

RESULTS AND DISCUSSION

For all tests the combined drug standard gave three distinct spots upon development. The solvent development system had been previously chosen after several different combinations of solutions were evaluated such as chloroform/ MeOH/NH (50:10:1), toluene/ethyl acetate/ammonia (8:4:1), etc. (Appendix 1) The solvent system, a variation of the ethyl acetate/methanol/ammonia(aq) solvent system, was chosen for its desired separation parameters of all the compounds. The compounds' Rf (the distance the sample migrates divided by the distance the solvent front migrates) were in the desired range of 0.2 - 0.8. This range was chosen to assure complete compound resolution from both the application position and the solvent front. The selected manufacturers, as expected for the same stationary phase, silica gel, had the same elution order of codeine, methadone, and cocaine. The differences were in the actual values of the area and Rf. The manufacturers' mean values of Rf and area for each drug are listed in Tables 1, 2, and 3, along with the standard deviation from the mean of the plates analyzed.

Table 1 - CHROMATOGRAPHIC REPRODUCIBILITY - CODEINE

Manufacturer	Mean Rf (% Standard deviation)	Mean Area (% Standard Deviation)
Alltech	0.22 (9%)	12204 (83%)
Analtech	0.17 (18%)	48017 (17%)
Baker	0.24 (8%)	10103 (14%)
E. Merck	0.30 (13%)	9187 (0.5%)
Whatman	0.23 (13%)	8416 (48%)

Table 2 - CHROMATOGRAPHIC REPRODUCIBILITY - METHADONE

Manufacturer	Mean Rf (% Standard deviation)	Mean Area (% Standard Deviation)
Alltech	0.54 (7%)	5930 (118%)
Analtech	0.32 (12%)	26332 (45%)
Baker	0.52 (10%)	3879 (3%)
E. Merck	0.69 (12%)	4949 (1%)
Whatman	0.49 (12%)	4064 (38%)

Table 3 - CHROMATOGRAPHIC REPRODUCIBILITY - COCAINE

Manufacturer	Mean Rf (% Standard deviation)	Mean Area (% Standard Deviation)
Alltech	0.69 (4%)	8939 (25%)
Analtech	0.60 (15%)	9799 (14%)
Baker	0.73 (9%)	10972 (4)
E. Merck	0.84 (5%)	14166 (1%)
Whatman	0.68 (9%)	11388 (28%)

TEST 1

Test 1 was performed using a mercury lamp at λ=254nm, as referenced in the literature[20]. The first test's statistical analysis yielded variance results for each manufacturer based on the linear model in Equation 1. The variances of area and Rf (Tables 4 - 9) are due to the differences between the plates, tracks, and the scans for each manufacturer. The Rf data shows that the variance of each manufacturer is consistently a result of individual plate differences of that manufacturer. The measured Rf is primarily dependent upon the particular plate chosen randomly from the stack, while the difference of Rf values between tracks and scans comprises only a small part of the total variance. In contrast, the area is

primarily dependent upon the track, i. e. the track placement on the plate, for all the manufacturers, except E. Merck. Non-homogenous layering of the stationary phase and surface irregularities are possible causes for this variance. The scan term shows that there can be significant variability with the repeated scans of a individual track in respect to area and in some cases Rf. These variances were a result of the inconsistent integration of the chromatograms brought about by noisy background, poor peak shape and incomplete peak resolution.

Table 4 - Rf Sources of Variances - Codeine

Manufacturer	%s Plate	%s Track	%s Scan
Alltech	75.8% (0.02)	21.6% (0.01)	2.6% (0)
Analtech	96.3% (0.03)	3.2% (0.01)	0.5% (0)
Baker	94.5% (0.02)	5.5% (0.01)	0% (0)
E. Merck	98.1% (0.04)	1.9% (0.01)	0% (0)
Whatman	72.5% (0.03)	26.0% (0.02)	1.5% (0)

%s is percentage of total variation of the mean Rf

Table 5 - Area Sources of Variances - Codeine

Manufacturer	%s Plate	%s Track	%s Scan
Alltech	50.2% (10231)	49.8% (10181)	0% (0)
Analtech	1.1% (5998)	97.1% (57170)	1.8% (7024)
Baker	3.8% (545)	68.5% (2307)	27.7% (1467)
E. Merck	9.2% (154)	89.8% (483)	1.0% (50)
Whatman	0% (0)	0% (0)	100% (4067)

%s is percentage of total variation of the mean Area

Table 6 - Rf Sources of Variances - Methadone

Manufacturer	%s Plate	%s Track	%s Scan
Alltech	52.5% (0.03)	47.3% (0.02)	0% (0)
Analtech	78.0% (0.04)	26.7% (0.02)	19.0% (0.02)
Baker	80.9% (0.05)	19.0% (0.02)	0% (0)
E. Merck	97.3% (0.04)	2.7% (0.01)	0% (0)
Whatman	86.3% (0.06)	12.7% (0.02)	1.0% (0.01)

%s is percentage of total variation of the mean Rf

Table 7 - Area Sources of Variances - Methadone

Manufacturer	%s Plate	%s Track	%s Scan
Alltech	0% (0.)	13.3% (2733)	86.7% (6980)
Analtech	8.3% (8075)	73.8% (24037)	17.9% (11829)
Baker	0% (0)	93.2% (475)	6.8% (126)
E. Merck	71.1% (1020)	28.8% (649)	0.1% (48)
Whatman	9.0% (541)	18.6% (774)	72.4% (1529)

%s is percentage of total variation of the mean Area

Table 8 - Rf Sources of Variances - Cocaine

Manufacturer	%s Plate	%s Track	%s Scan
Alltech	56.1% (0.02)	42.7% (0.02)	1.2% (0)
Analtech	91.7% (0.10)	8.3% (0.03)	0 % (0)
Baker	74.4% (0.05)	25.5% (0.03)	0.1% (0)
E. Merck	98.5% (0.05)	1.5% (0.01)	0% (0)
Whatman	82.0% (0.06)	17.0% (0.03)	1.0% (0.06)

%s is percentage of total variation of the mean Rf

Table 9 - Area Sources of Variances - Cocaine

Manufacturer	%s Plate	%s Track	%s Scan
Alltech	0% (0)	52.9% (2404)	47.1% (2270)
Analtech	70.6% (3426)	17.4% (1703)	12.0% (1413)
Baker	33.8% (1631)	64.0% (2245)	2.2% (419)
E. Merck	93.3% (5138)	6.6% (1382)	0.1% (166)
Whatman	0% (0)	0% (0)	100% (3193)

%s is percentage of total variation of the mean Rf

The second statistical test compared the results of Test 1 by manufacturer. The specific value used for comparison was the p-value, the result of the F-test. The p-value showed the significance of the test, i. e. a lower p-value showed a more significant difference between the two manufacturers. Tables 10 - 15 list the p-values for Rf and area F-tests combined with least squares mean test, as described in the statistics Test 2. The tables shows p-values based on the particular drug although this has little effect on the Rf results. The variances seen by the drug for E. Merck's and Analtech's plates display a significant difference between each other as well as with the other three manufacturers' plates. Analtech's plates have comparatively low peak area and low Rf's for the three compounds. The peak area for codeine and methadone differ significantly from the other manufacturers, which, amongst themselves, fail to show a significant difference. On E. Merck plates, cocaine's results for both area and Rf vary significantly from Baker, Whatman and Alltech plates (which are similar to each other).

Table 10 - Rf RESULTS FROM LEAST SQUARES MEAN TEST - CODEINE

RF:	Alltech	Analtech	Baker	E. Merck	Whatman
Alltech	-	0.0219	0.4326	0.0015	0.8316
Analtech	0.0219	-	0.0043	0.0001	0.0142
Baker	0.4326	0.0043	-	0.0078	05640
E. Merck	0.0015	0.0001	0.0078	-	0.0023
Whatman	0.8316	0.0142	0.5640	0.0023	-

Table 11 - Rf RESULTS FROM LEAST SQUARES MEAN TEST - METHADONE

RF:	Alltech	Analtech	Baker	E. Merck	Whatman
Alltech	-	0.0002	0.5877	0.0019	0.2095
Analtech	0.0002	-	0.0004	0.0001	0.0020
Baker	0.5877	0.0004	-	0.0006	0.4542
E. Merck	0.0019	0.0001	0.0006	-	0.0002
Whatman	0.2095	0.0020	0.4542	0.0002	-

Table 12 - Rf RESULTS FROM LEAST SQUARES MEAN TEST - COCAINE

RF:	Alltech	Analtech	Baker	E. Merck	Whatman
Alltech	-	0.0267	0.3364	0.0021	0.8434
Analtech	0.0267	-	0.0052	0.0001	0.367
Baker	0.3364	0.0052	-	0.0152	0.2513
E. Merck	0.0021	0.0001	0.0152	-	0.0014
Whatman	0.8434	0.0367	0.2513	0.0014	-

Table 13 - AREA RESULTS FROM LEAST SQUARES MEAN TEST - CODEINE

Area:	Alltech	Analtech	Baker	E. Merck	Whatman
Alltech	-	0.0050	0.8497	0.7856	0.7324
Analtech	0.0050	-	0.0034	0.0028	0.0024
Baker	0.8497	0.0034	-	0.9341	0.8789
E. Merck	0.7856	0.0028	0.9341	-	0.9445
Whatman	0.7324	0.0024	0.8789	0.9445	-

Table 14 - AREA RESULTS FROM LEAST SQUARES MEAN TEST - METHADONE

Area:	Alltech	Analtech	Baker	E. Merck	Whatman
Alltech	-	0.0234	0.7510	0.8792	0.7528
Analtech	0.0234	-	0.0122	0.0172	0.0123
Baker	0.7510	0.0122	-	0.8683	0.9981
E. Merck	0.8792	0.0172	0.8683	-	0.8702
Whatman	0.7528	0.0123	0.9981	0.8702	-

Table 15 - AREA RESULTS FROM LEAST SQUARES MEAN TEST - COCAINE

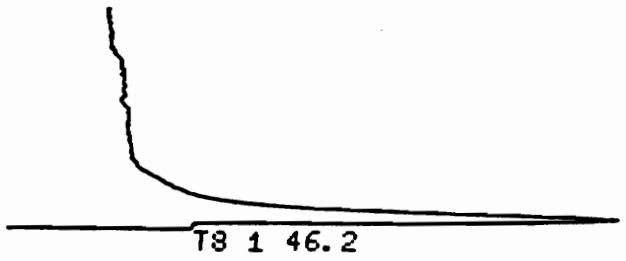
Area:	Alltech	Analtech	Baker	E. Merck	Whatman
Alltech	-	0.2868	0.4374	0.5810	0.3519
Analtech	0.2868	-	0.0765	0.0065	0.0566
Baker	0.4374	0.0765	-	0.2293	0.8727
E. Merck	0.5810	0.0065	0.2293	-	0.2928
Whatman	0.3519	0.0566	0.8727	0.2928	-

TEST 2

Signal to noise (S/N) tests started with analyzing for the optimum wavelength by using a deuterium lamp instead of the mercury lamp as in Test 1. (Figures 2 through 6) The deuterium lamp allowed a full spectrum of wavelengths to be used rather than just the mercury spectrum bands. (The deuterium lamp was not available for previous studies.) The highest sensitivity was achieved at λ=230nm. Since it was not thought that the previous test results would significantly change if analyses were done at λ=230nm instead of λ=254nm, previous tests were not repeated. Baker and E. Merck plates were used for this test because these two manufacturers showed the lowest background noise and best overall peak shape for all compounds analyzed. The S/N test chromatograms are seen in figures 7 and 8. The S/N ratio was determined by dividing the height of the peak by the average height deviation of the entire chromatogram's baseline, i. e. the average noise. The S/N ratios are listed in Table 16. E. Merck have larger S/N ratios primarily due to lower baseline noise since the peak heights of each compound for both manufacturers' plates are almost the same.

Table 16 - Signal To Noise Ratios

	E. Merck	Baker
Codeine	40.2	16.7
Methadone	20.8	7.1
Cocaine	32.8	12.4

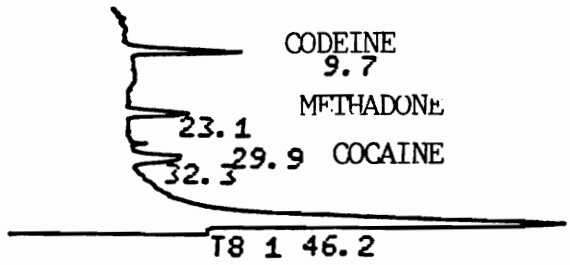


10-26-87 08:21:11

FILE 1. METHOD 0. RUN 1 INDEX 1

PEAK#	AREA%	RT	AREA BC
1	100.	46.2	59226 01
TOTAL	100.		59226

Figure 2 - Blank Chromatogram

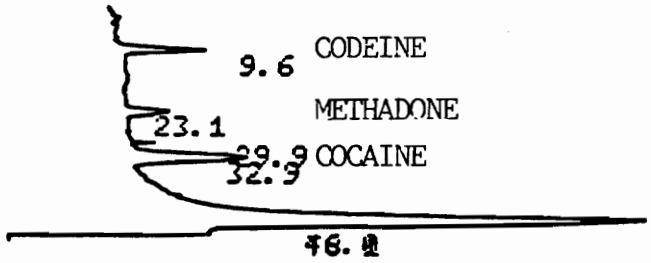


10-26-87 08:33:08

FILE 1. METHOD 0. RUN 10 INDEX 10

PEAK#	AREA%	RT	AREA BC
1	12.781	9.7	10565 01
2	8.051	23.1	6655 01
3	0.726	29.9	600 01
4	9.029	32.3	7463 01
5	69.413	46.2	57376 01
TOTAL	100.		82659

Figure 3 - Test Wavelength 220 nm

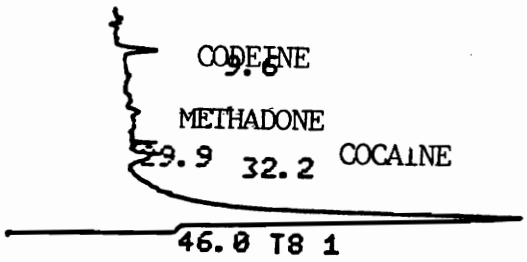


10-26-87 08:31:55

FILE 1. METHOD 0. RUN 9 INDEX 9

PEAK#	AREA%	RT	AREA	BC
1	8.155	9.6	8094	01
2	4.967	23.1	4930	01
3	0.712	29.9	707	01
4	18.631	32.9	18491	01
5	67.534	46.	67027	01
TOTAL	100.		99249	

Figure 4 - Test Wavelength 230 nm



10-26-87 08:22:42

FILE 1. METHOD 0. RUN 2 INDEX 2

PEAK#	AREA%	RT	AREA	BC
1	4.339	9.6	3338	01
2	0.837	29.9	644	01
3	0.667	32.2	513	01
4	94.157	46.	72439	01
TOTAL	100.		76934	

Figure 5 - Test Wavelength 254 nm

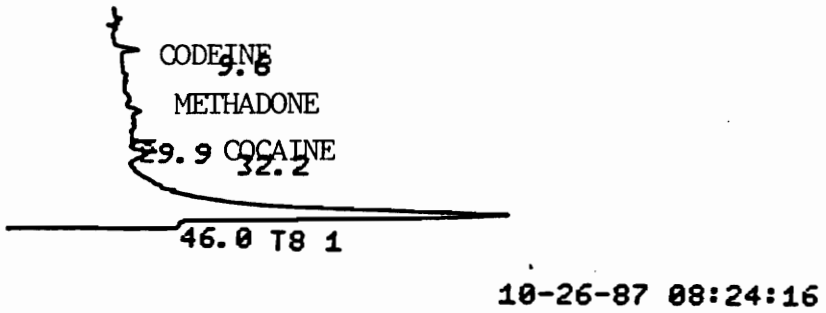


Figure 6 - Test Wavelength 260 nm

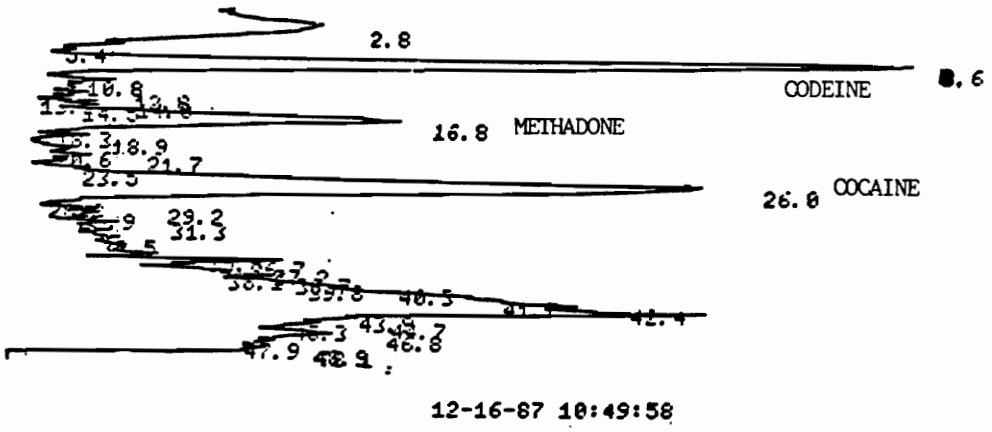
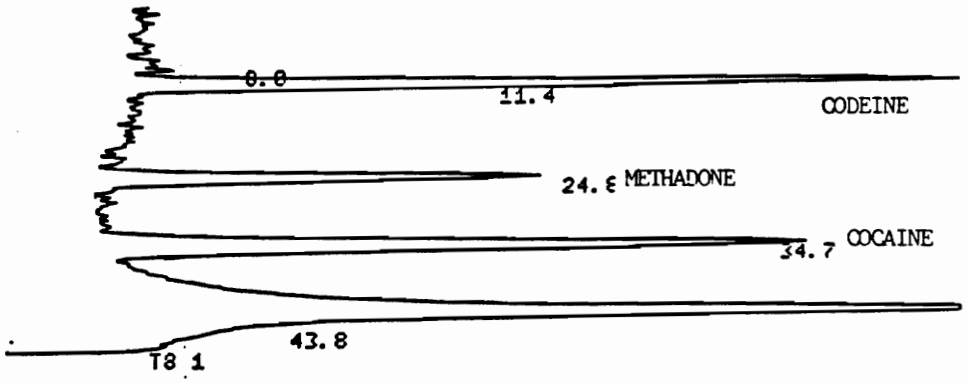


Figure 7 - Baker Plate Signal/Noise Chromatogram
(not to scale)



12-16-87 11:38:58 CH= "A" PS= 1.

FILE 1. METHOD 0. RUN 44 INDEX 44

PEAK#	AREA%	RT	AREA BC
1	0.954	8.	2862 02
2	20.523	11.4	61578 03
3	11.201	24.8	33607 01
4	24.416	34.7	73261 01
5	42.907	43.8	128741 01
TOTAL	100.		300049

Figure 8 - E. Merck Plate Signal/Noise Chromatogram
(not to scale)

TEST 3

E. Merck plates were chosen for the single manufacturer scanning of one band 10 times because of the higher signal to noise ratio and well defined peak shape of all compounds analyzed, the methadone and cocaine peak shape varied widely for other manufacturers.. The results are given in % standard deviation of area in Table 17.

Table 17 - Results of Repeated Scans of E. Merck Plates

Drug	%s
Codeine	0.49
Methadone	0.11
Cocaine	4.59

%s is percent standard deviation of area

Results were analyzed by the statistical test 3. The overall results (Table 18) display expected results from this test. The Rf is dependent on the drug, the concentration, and the plate(drug) interaction. The area is dependent on the drug, the concentration, and the drug(concentration) interaction.

Table 18 - Overall Results For Single Plate Test

Rf : dependent on
1) drug
2) concentration
3) plate-drug interaction

AREA: dependent on
1) drug
2) concentration
3) drug-concentration interaction

The codeine results (Table 19) and methadone results (Table 20) show that the Rf means were constant for two concentrations. The area of each drug increases almost three times with a 3-fold

increase of the concentration, giving a linear relationship of 1:1. The F-test results displayed a significant area dependence with respect to the concentration, as was expected for the interaction between a given plate and concentration on a specific day.

Table 19 - Codeine Results for E. Merck

Means:	Rf	0.02	
	Area	8090 (100ng)	
		22011 (300ng)	
p-value			
Dependent on		1) concentration	0.0001
		2) plate-concentration(day)	0.0015
		3) day-concentration(day)	0.0307

Table 20 - Methadone Results for E. Merck

Means:	Rf	0.48	
	Area	5075 (100ng)	
		14395 (300ng)	
p-value			
Dependent on		1) concentration	0.0001
		2) day-concentration	0.0071

The cocaine results (Table 21) are slightly different from codeine and methadone in that although the Rf mean remains constant with both concentrations, the area doubles with a 3-fold increase of the concentration giving a non-linear relationship. Absorbance measurements frequently display non-linear relationships[22]. The area displays a dependence on only the concentration and not on the specific day that the tests were analyzed.

Table 21 - Cocaine Results for E. Merck

Means: Rf 0.69
Area 25130 (100ng)
48516 (300ng)

p-value
Dependent on 1) concentration 0.0004

The area concentration effects were determined by summarizing all drug results, as seen in Table 22. At 100ng, the area is dependent on the particular drug and its sensitivity to UV activity (λ=230nm). At 300ng, the area is not only effected by the drug, but also by the day spectrophotometrically scanned and the plate(drug) interaction. The number of days scanned from when the plates were spotted and developed is significant because as time passed the samples faded, i.e. lost sample concentration. The higher concentrations on some plates could have been displaying sample overload, by losing peak area as compared to the lower concentration samples.

Table 22 - CONCENTRATION DEPENDENT RESULTS

AREA:

1) 100ng
dependent on drug: p-value = 0.0003

2) 300ng
dependent on drug: p-value = 0.0001
dependent on day: p-value = 0.0001
dependent on plate-drug
interaction p-value = 0.0007

Test 4

Example chromatograms for the plate manufacturers are shown in Figures 9 - 13 (λ=230, 100 ng). The calibration curve results were achieved by plotting the area of the drug versus its concentration. The calibration curve plots (Figures 14 - 28) a linear equation line drawn through the data points. If a point to point line is drawn the curve falls downward slightly at the higher concentrations showing that

plate overload (concentration) may be a contributing factor, although absorbance measurements also tend to display this effect. Using test curves for the determination of the "unknown" concentration of the 150ng sample, the resulting concentrations for each drug versus their respective manufacture are listed in Table 23. The Baker plates demonstrate the closest accuracy with 2 -7% error range around the known concentration. E. Merck plates followed with a 3 - 11% error range. Alltech with its ranges of 0.7 - 25%, showed the largest deviation from the true value.

Table 23 - CALIBRATION CURVE (UNKNOWN = 150ng)

MANUFACTURER	CODEINE	METHADONE	COCAINE
ALLTECH	151 (0.7%)	113 (25%)	155 (3%)
ANALTECH	123 (18%)	135 (10%)	130 (13%)
BAKER	143 (5%)	139 (7%)	153 (2%)
E. MERCK	134 (11%)	146 (3%)	140 (7%)
WHATMAN	126 (16%)	169 (13%)	169 (13%)

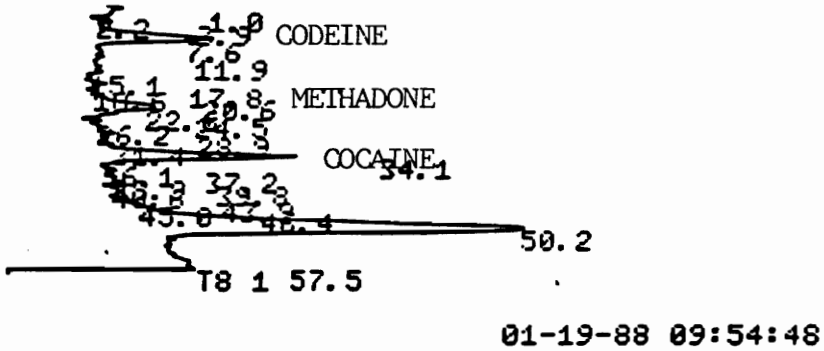


Figure 9 - Alltech, 100 ng Chromatogram

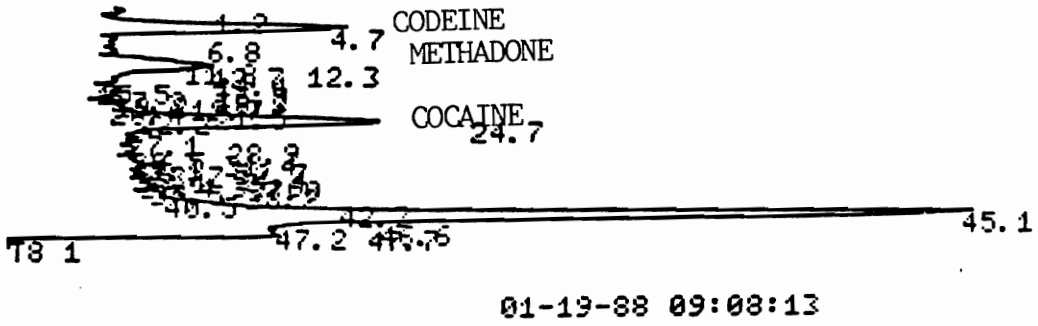


Figure 10 -Analtech, 100 ng Chromatogram

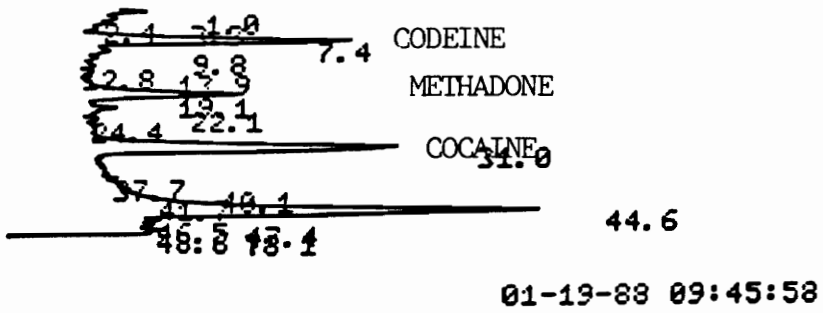


Figure 11 -Baker, 100 ng Chromatogram

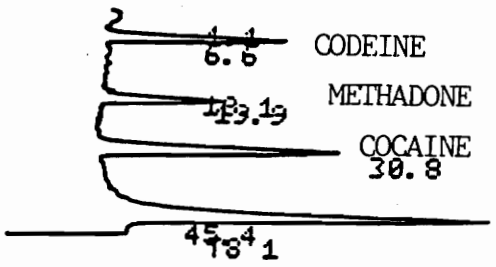


Figure 12 - E. Merck, 100 ng Chromatogram

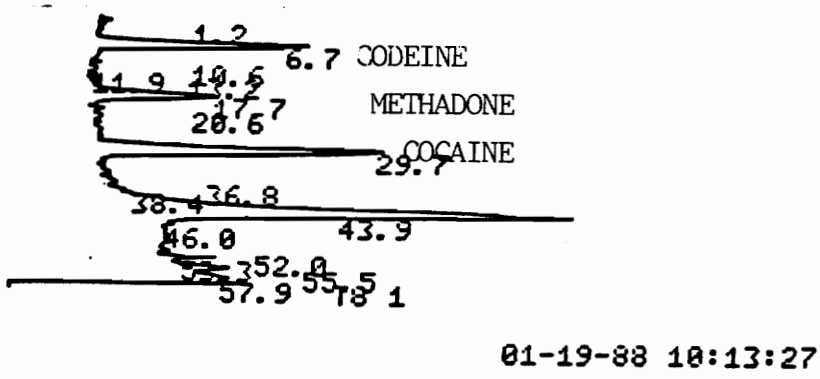


Figure 13 -Whatman, 100 ng Chromatogram

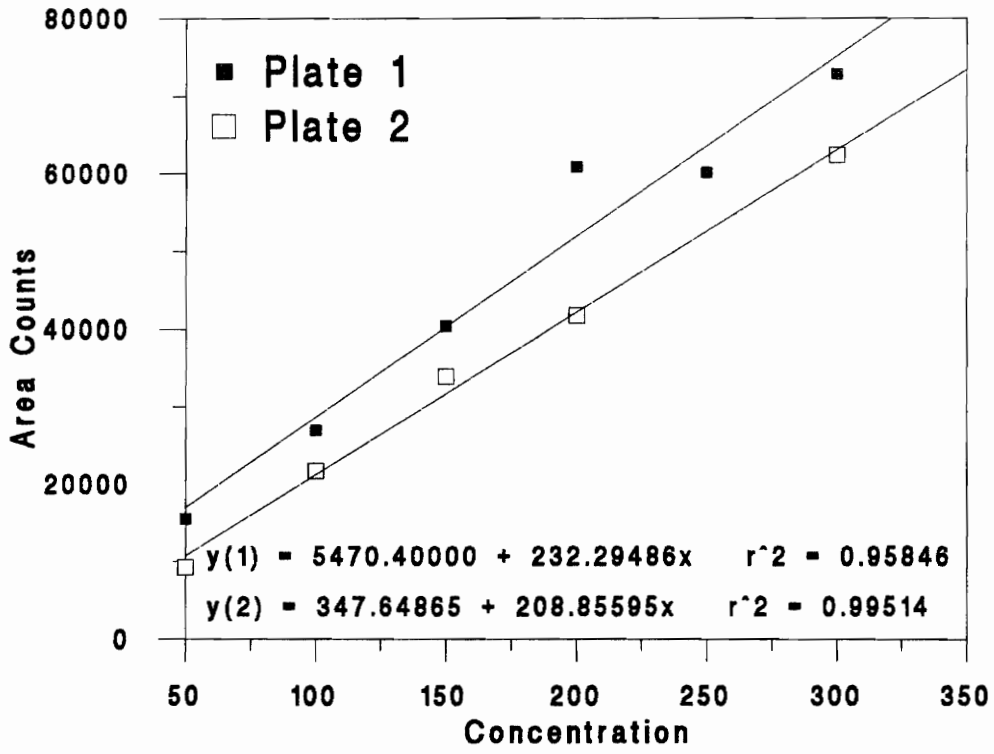


Figure 14 - Calibration Curve for Codeine on Alltech Plates

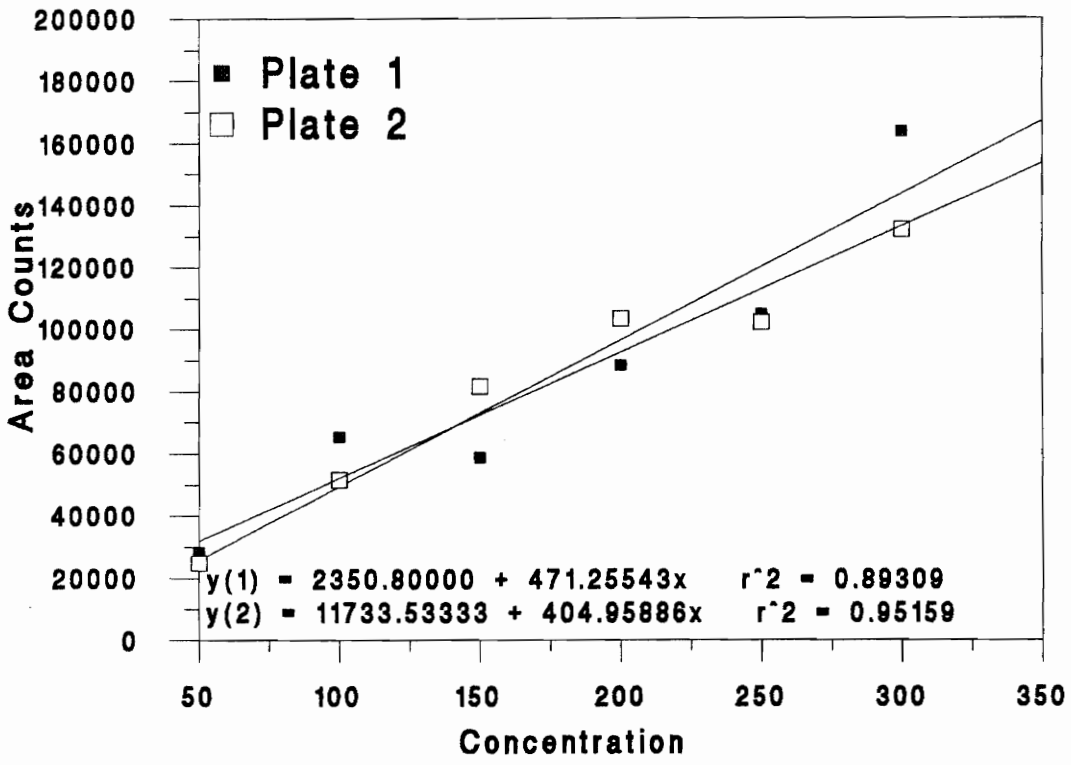


Figure 15 - Calibration Curve for Codeine on Analtech Plates

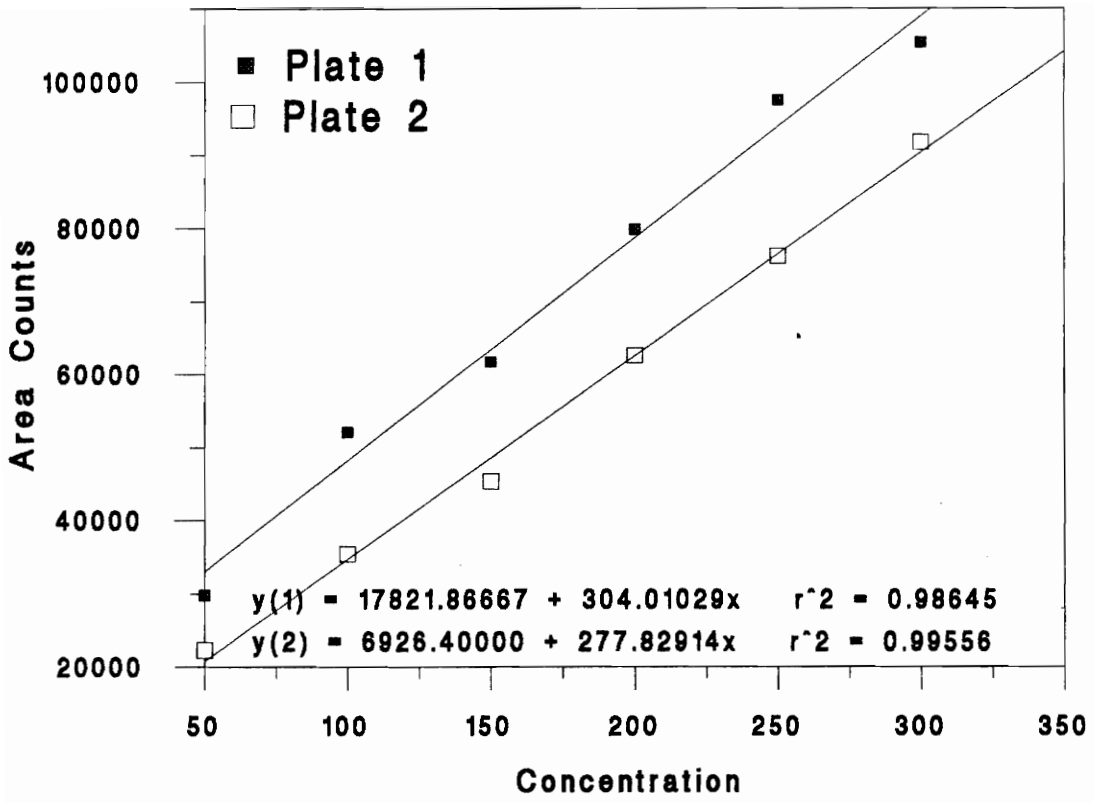


Figure 16 - Calibration Curve for Codeine on Baker Plates

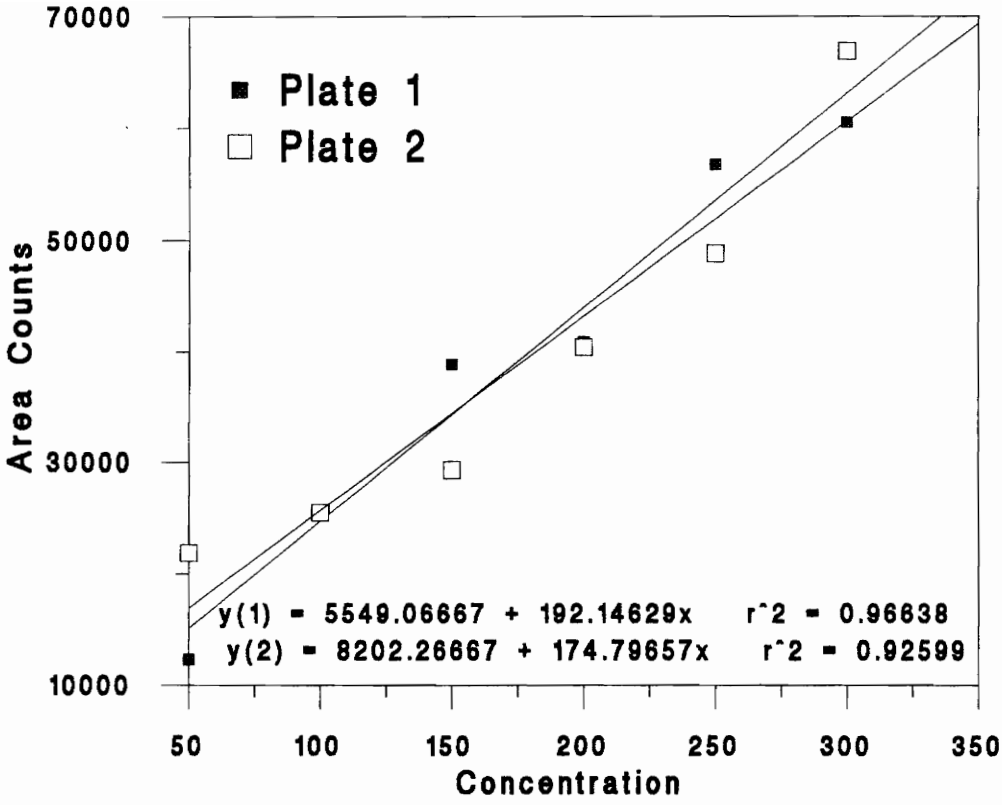


Figure 17 - Calibration Curve for Codeine on E. Merck Plates

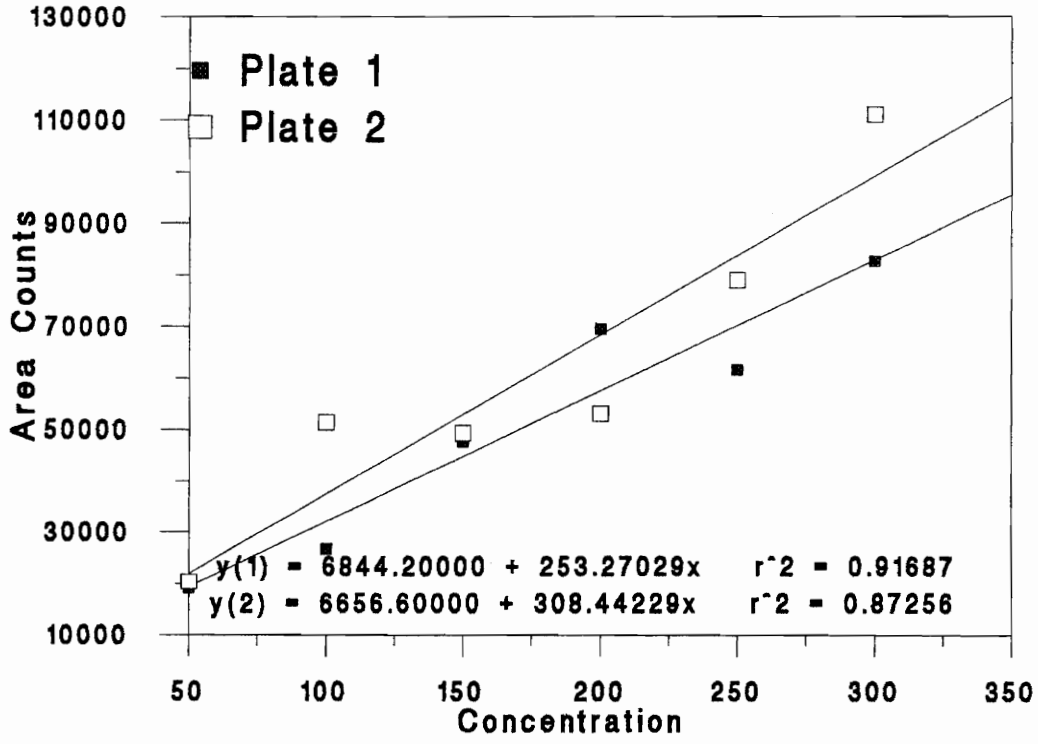


Figure 18 - Calibration Curve for Codeine on Whatman Plates

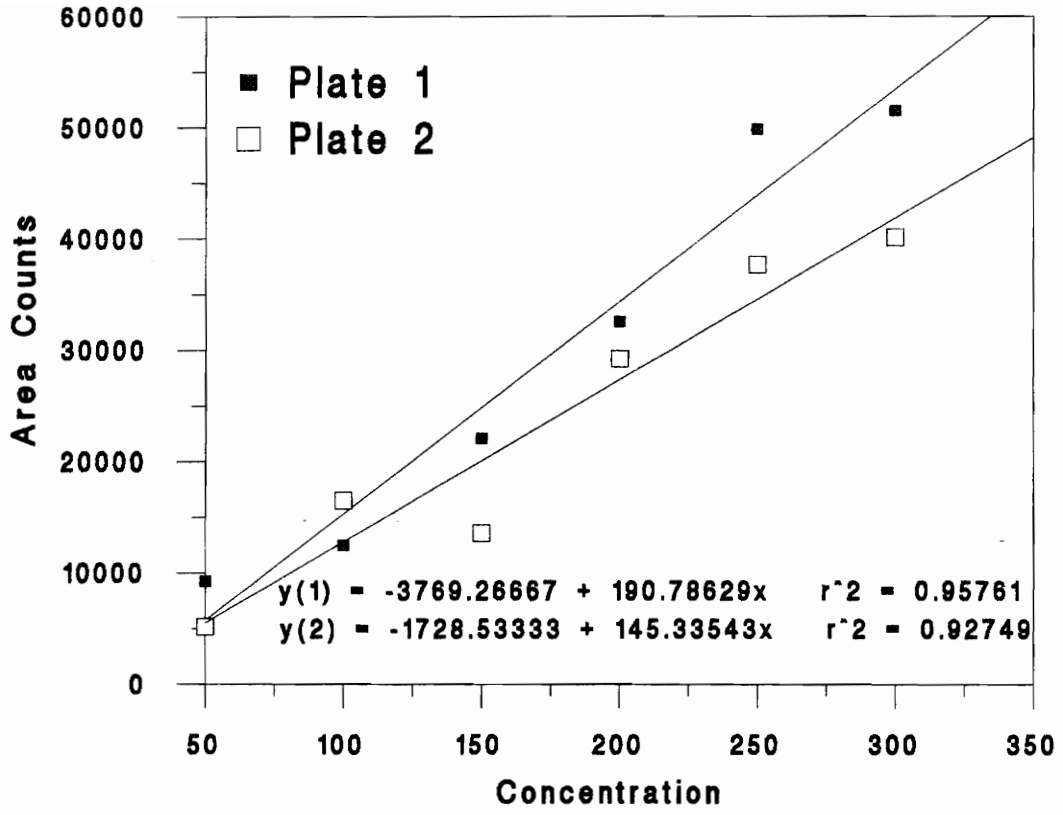


Figure 19 - Calibration Curve for Methadone on Alltech Plates

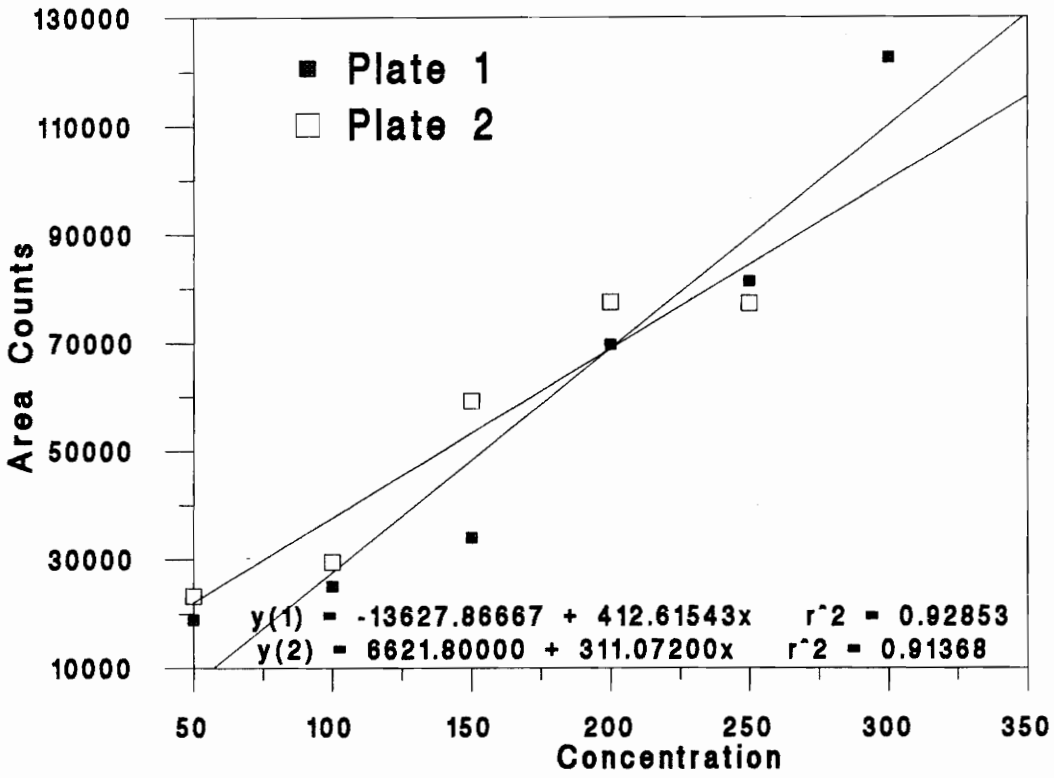


Figure 20 - Calibration Curve for Methadone on Analtech Plates

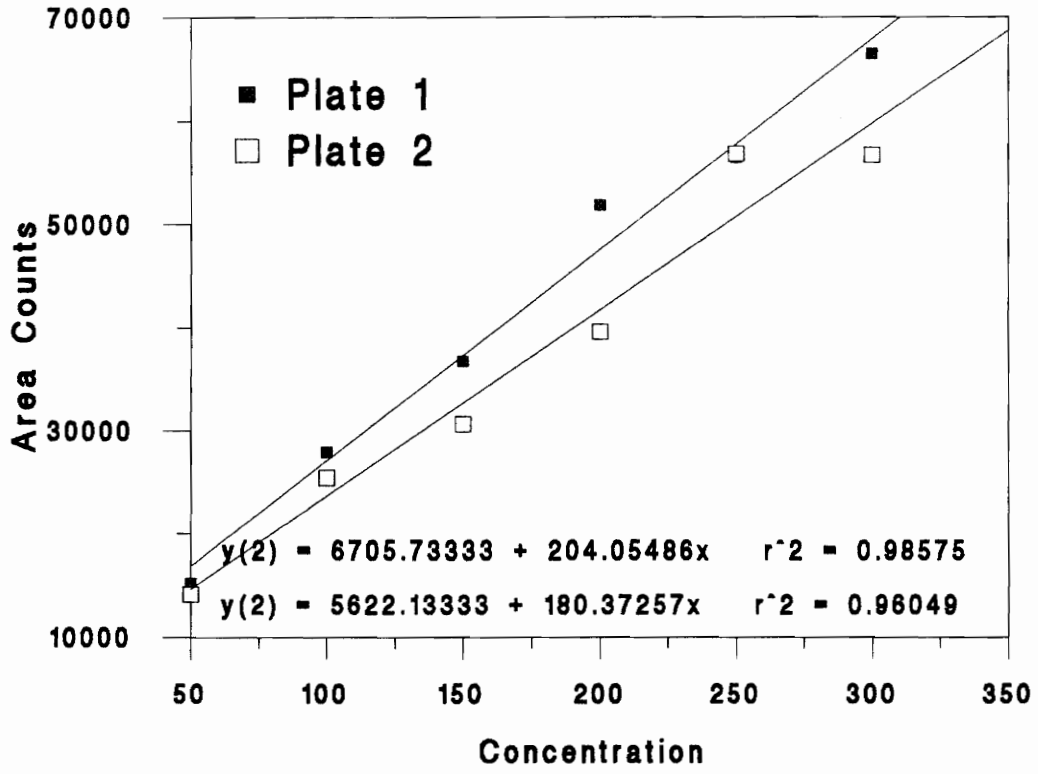


Figure 21 - Calibration Curve for Methadone on Baker Plates

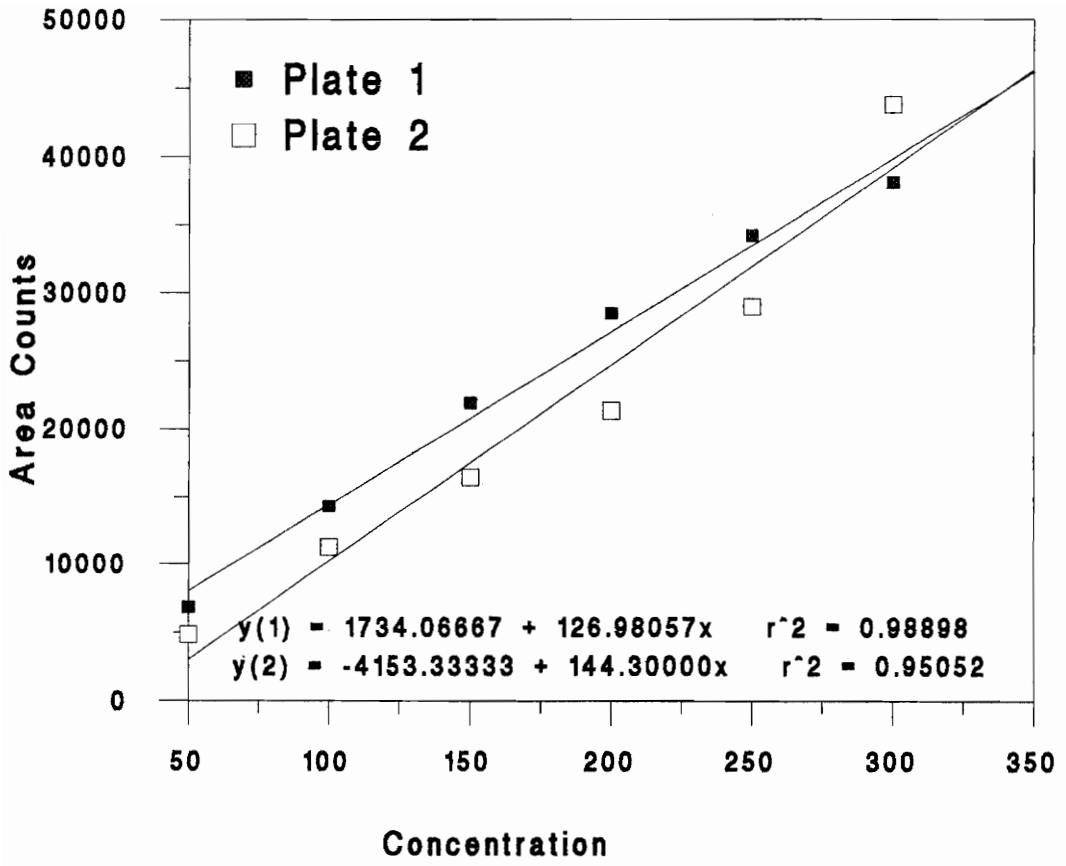


Figure 22 - Calibration Curve for Methadone on E. Merck Plates

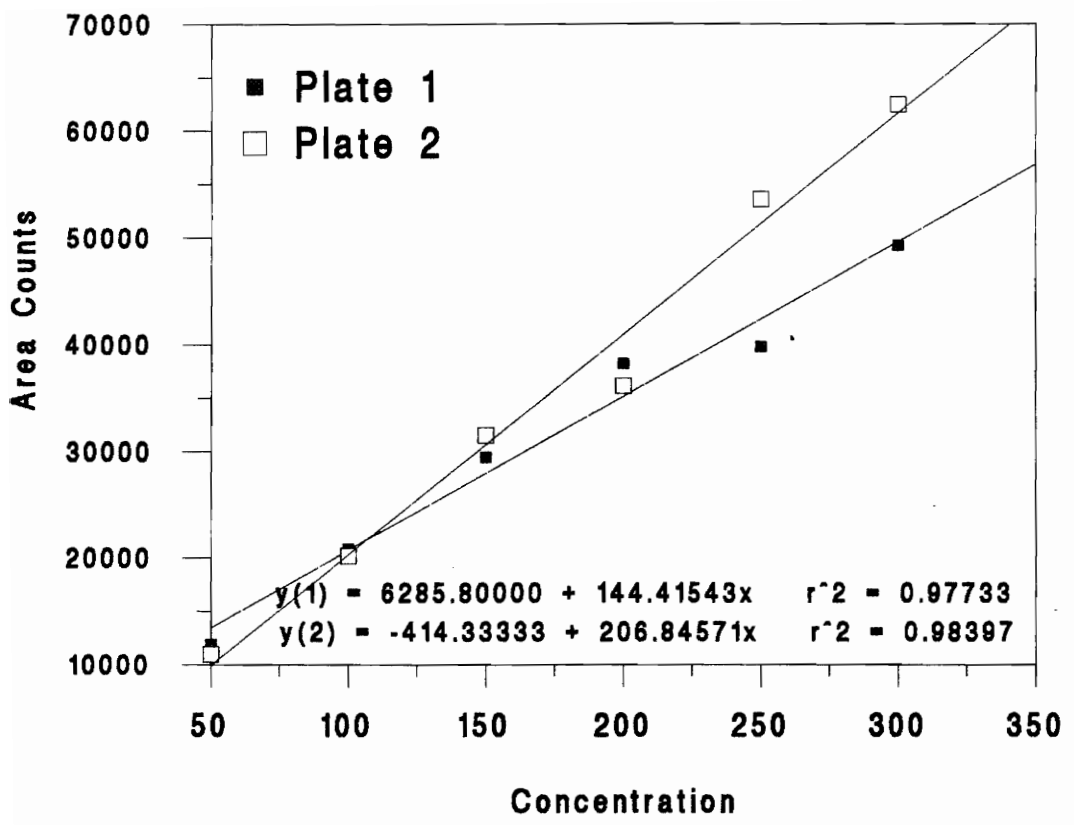


Figure 23 - Calibration Curve for Methadone on Whatman Plates

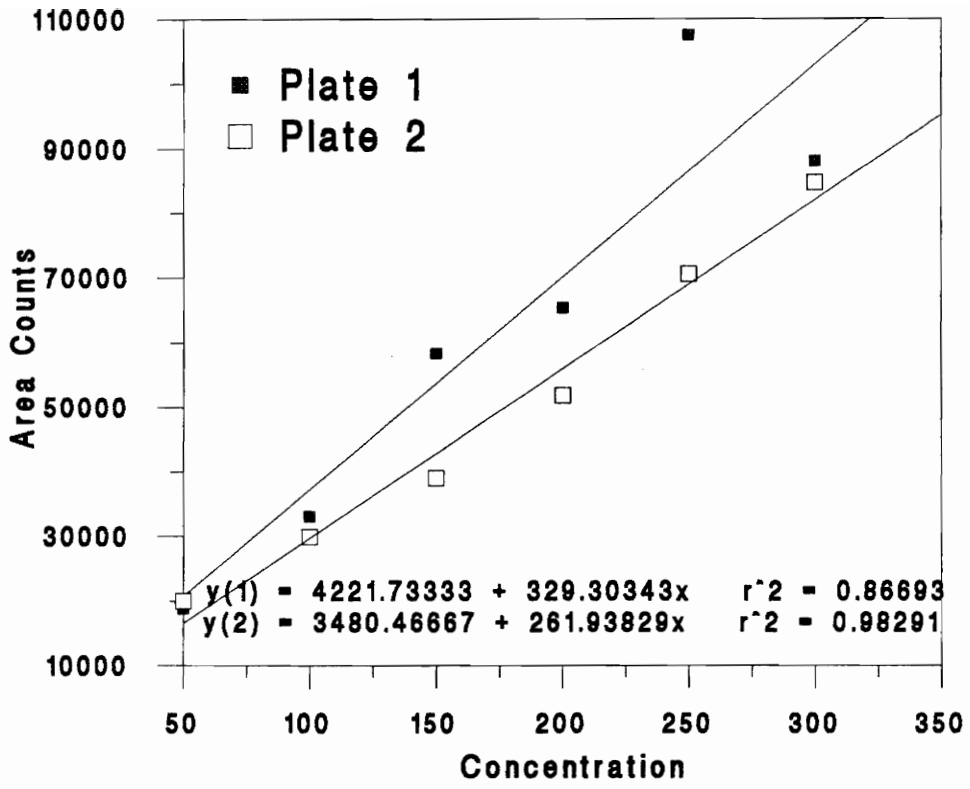


Figure 24 - Calibration Curve for Cocaine on Alltech Plates

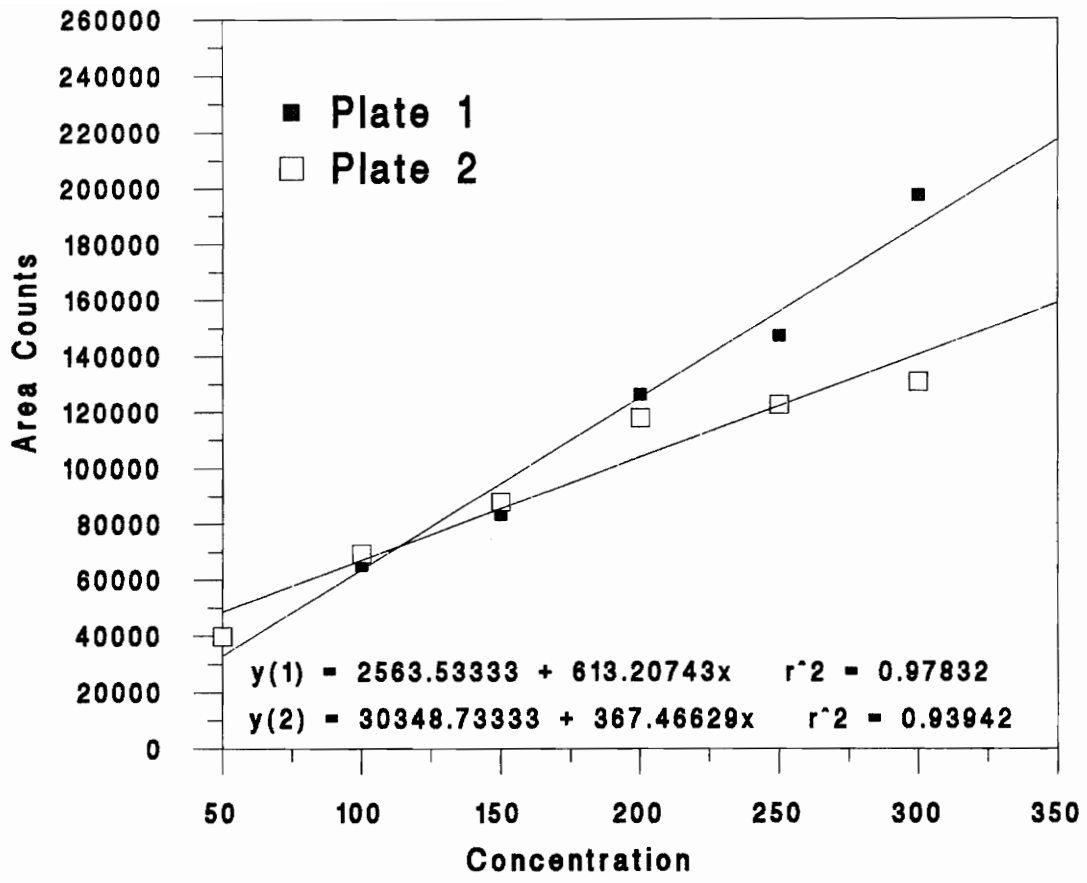


Figure 25 - Calibration Curve for Cocaine on Analtech Plate

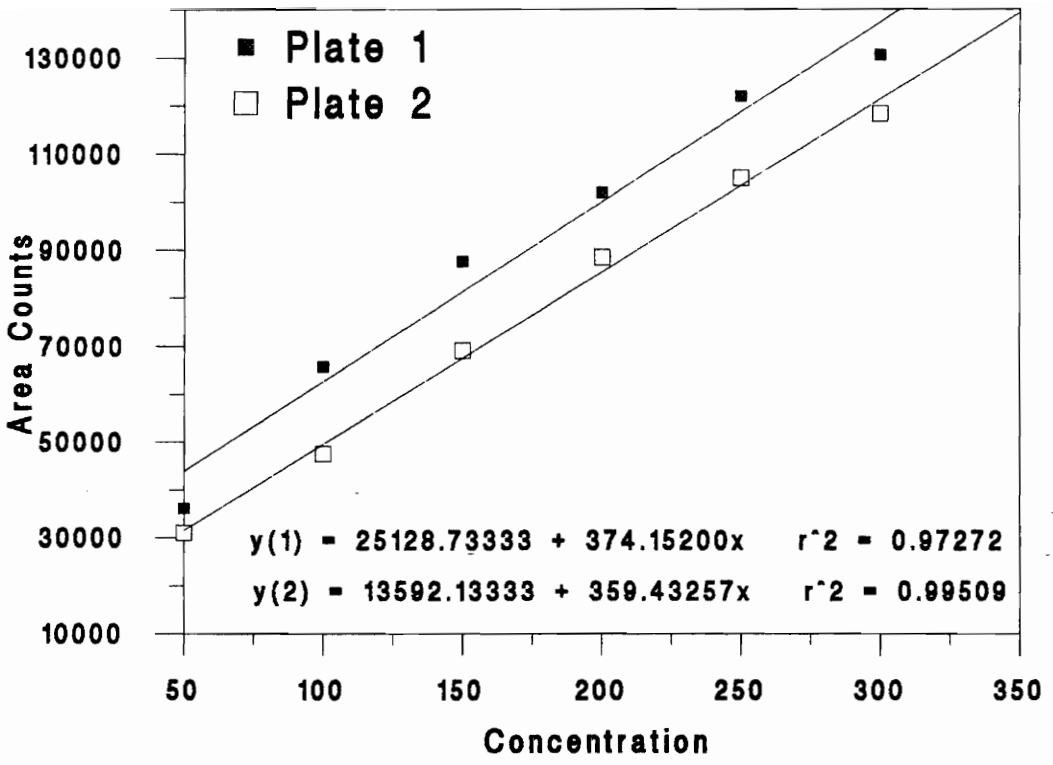


Figure 26 - Calibration Curve for Cocaine on Baker Plates

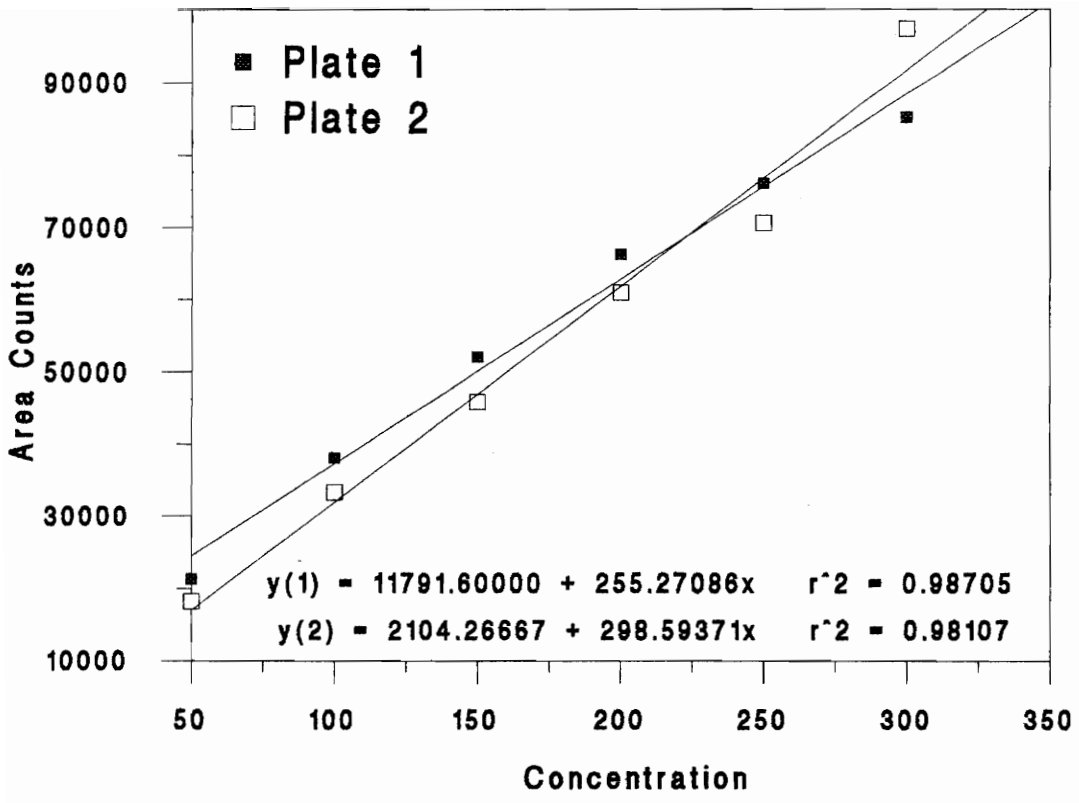


Figure 27 - Calibration Curve for Cocaine on E. Merck Plates

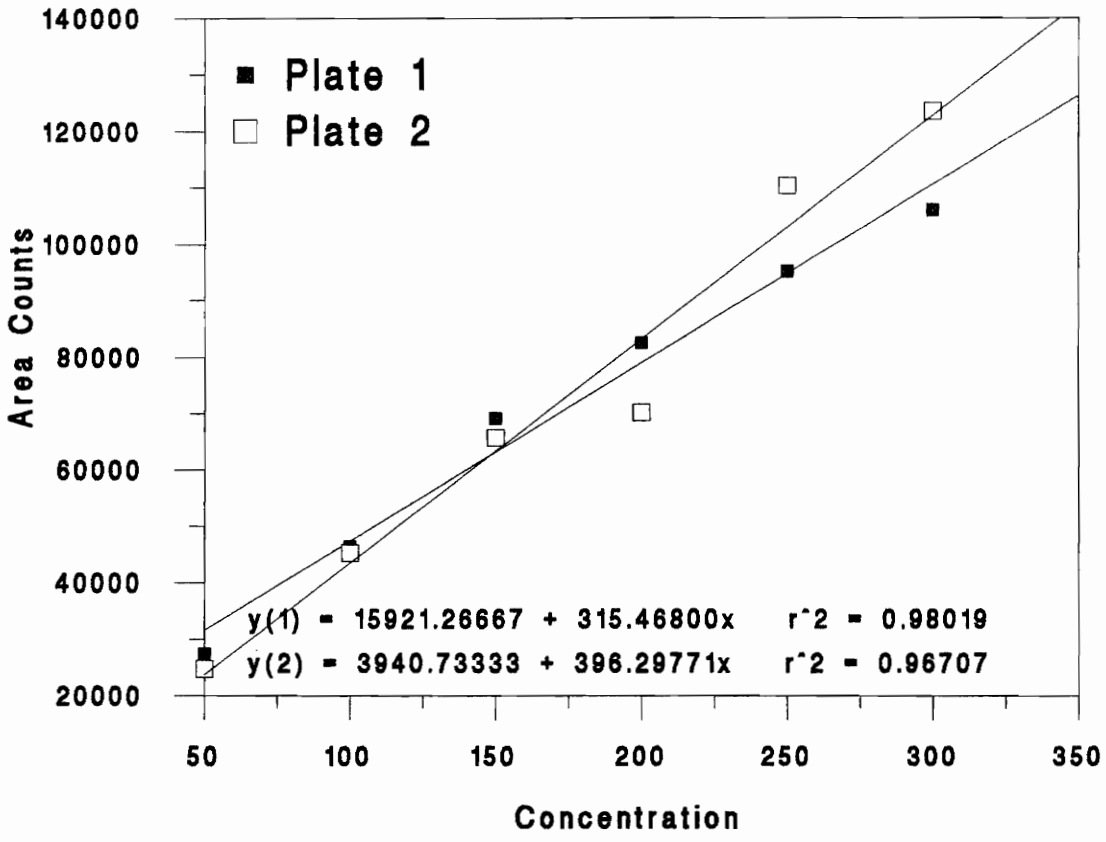


Figure 28 - Calibration Curve for Cocaine on Whatman Plates

Overall Results

When all the data is analyzed, several conclusions can be drawn. First, the resultant deviation from the mean of both the Rf and area were small for only two manufacturers, and are scattered a great deal for the other three manufacturers. The use of a single manufacturer's plate will yield more reliable and reproducible results for testing because a change in manufacturer could inaccurately display a loss in sensitivity and/or an increase in background noise.

Second, although one manufacturer (E. Merck) gives better reproducibility, not all sources of error and deviations are eliminated. The results show that for all manufacturers' plates a large source of error was present in the irregularity of the plate's surface, i. e. cracks and pits in the silica gel layer. To correct for this, calibration curves must be run using a series of concentrations on each plate, since each plate's linearity curve varies slightly. A general calibration curve test plate should be made for each sample to test linear range and to prevent possible overloading of the plate.

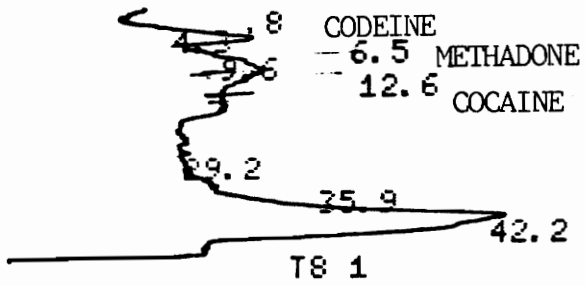
Third, the linearity of the calibration curve data is not seen for all manufacturers, as some show a leveling off at the higher concentrations. The manufacturers' plates which do not level off for any drug tested are Alltech, Baker, and E. Merck. Whatman and Analtech plates gave a fairly linear calibration curve for cocaine and codeine; methadone demonstrated the most erratic behavior. Possibly the branched chain structure was adsorbed to the surface differently than the other compounds which are cyclic, or the more basic character of methadone was seen.

The instrumentation contributed minor sources of error. The band applicator, which gave a smaller deviation in both area and Rf, can be tuned to give the least possible broadening of the initial application spot to reduce the deviations. Proper maintenance of the syringe and the syringe head can also help to reduce these deviations. The densitometer should be aligned with the band applicator to remove any possible error resulting from the misreading of the tracks. The densitometer had very little error associated with its general operation, however, the lamp itself can be a primary source of error. The

choice of the lamp, whether deuterium or mercury, plays a major role in the quality of the chromatogram. The mercury lamp only allows the use of its given lines, whereas, deuterium allows use of all spectral wavelengths. With the drugs used in the experiment, there was no mercury lamp wavelength line that coincided with an absorption maxima. Therefore, the deuterium lamp was found to be the better choice. Another possible source of error is the lifetime of the lamp, as a great deal of noise is present with older lamps.

Noise errors present themselves in several forms on the individual plates. Each plate used must be carefully examined for major surface irregularities before and after prewashing. Surface irregularities of concern were scratches, pitting or flaking of the stationary phase. Upon visual examination several of the plates were rejected before prewashing. Visual inspection under ultraviolet light (after prewashing), showed Analtech and Whatman plates had dark spots on the surface. These spots could have been unwashed impurities and/or large spaces between the silica gel particles as caused by the layering process. These dark spots were probably a large part of high background noise which was not seen in Baker, E. Merck or Alltech. Analtech plates had surface cracking while drying following solvent development. The cracking could be due to the thinner layer of this plate, 150μg rather than 200μg. These cracks could account for the increased noise, lower peak area counts, and larger plate variations seen for Analtech as compared to the other manufacturers.

The chromatographic resolution of Analtech plates was poor and inconsistent for both methadone and cocaine. (Figures 29 & 30, also Figure 10). They varied from barely coeluting to complete resolution. Codeine's Rf value was low (.1) and not in the desired Rf range (0.2 - 0.8). This solvent system, which worked well with the other manufacturers, could possibly be reworked to optimize the results on Analtech plates and this might affect the resolution problems.



08-22-87 09:46:57

FILE 1. METHOD 0. RUN 13 INDEX 13

PEAK#	AREA%	RT	AREA BC
1	7.955	0.8	22586 02
2	4.708	4.3	13369 02
3	14.879	6.5	42246 02
4	5.081	9.6	14428 02
5	15.186	12.6	43118 03
6	0.108	29.2	308 01
7	1.563	35.9	4438 02
8	50.52	42.2	143444 03
TOTAL	100.		283937

Figure 29 - Analtech Chromatogram with Incomplete Resolution of Methadone and Cocaine

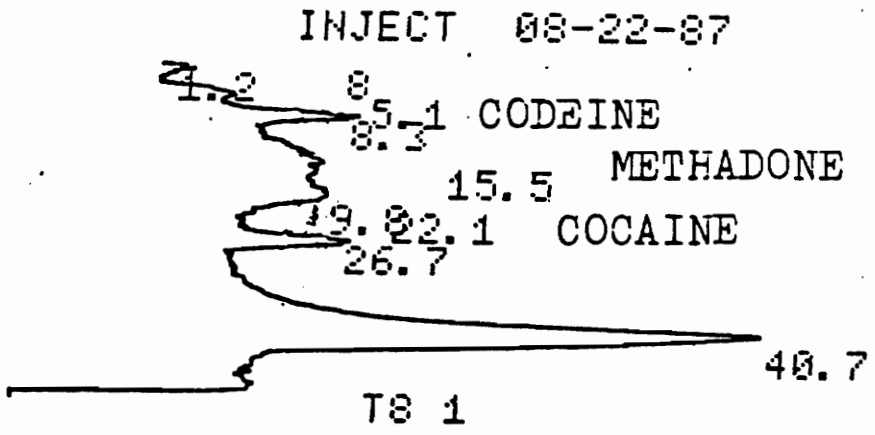


Figure 30 - Analtech Chromatogram with Band Broadening

With respect to Alltech's and Whatman's plates, their reliability depended upon the drug as well as its concentration. Both manufacturers' plates had a significant amount of background noise. (Figures 31 & 32) Whatman's plates results were reliable for all concentrations of cocaine, but only for 200μg of methadone, and not at all for codeine. Alltech's plates results were reliable for all drugs under 200μg with little variation, but over 200μg the deviations were significant, possibly due to sample overload. The chromatographic separation was adequate yet band broadening was seen in the low concentrations, particularly methadone. (Figure 31).

For overall performance, two plates manufacturers, Baker (Figure 33) and E. Merck (Figure 34) gave results far superior to the others in their reproducibility. Not only did they have lower background noise, but their plate surface error was the lowest, indicating that their layering methods are more uniform and reproducible. Baker plates gave the most linear calibration curves for the three drugs tested and the closest accuracy for the true value of the "unknown" test. E. Merck plates showed non-linearity in its calibration curve for codeine, possibly attributed to the low Rf value that was observed on some of the plates. Both manufacturers' products show excellent chromatographic resolution of all the drugs.

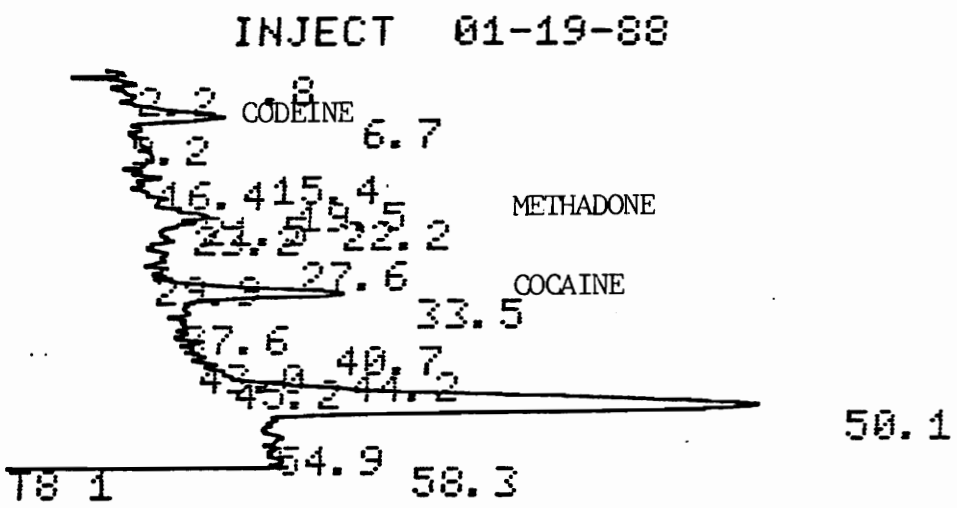


Figure 31 - Alltech Chromatogram
(not to scale)

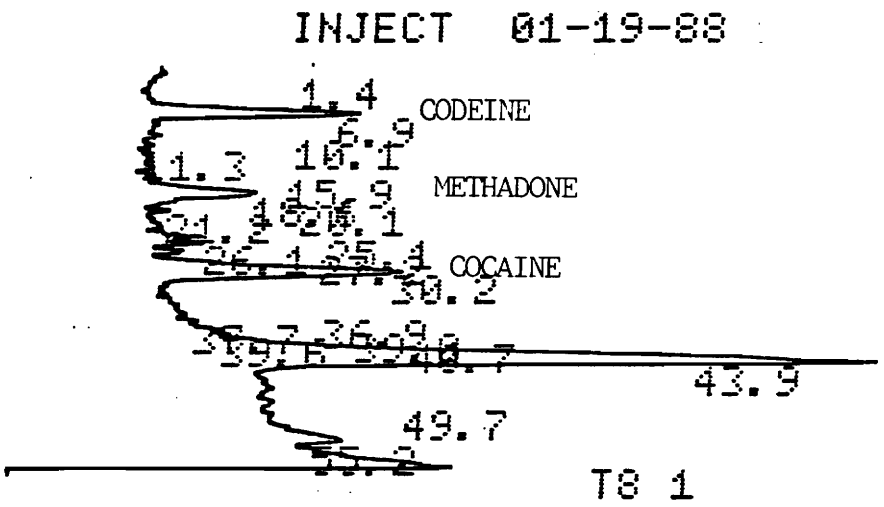


Figure 32 - Whatman Chromatogram
(not to scale)

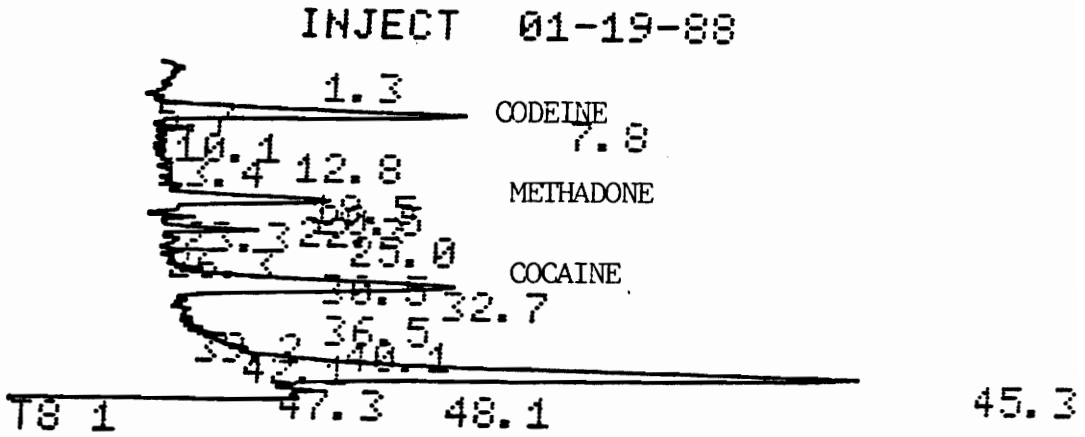


Figure 33 - Baker Chromatogram
(not to scale)

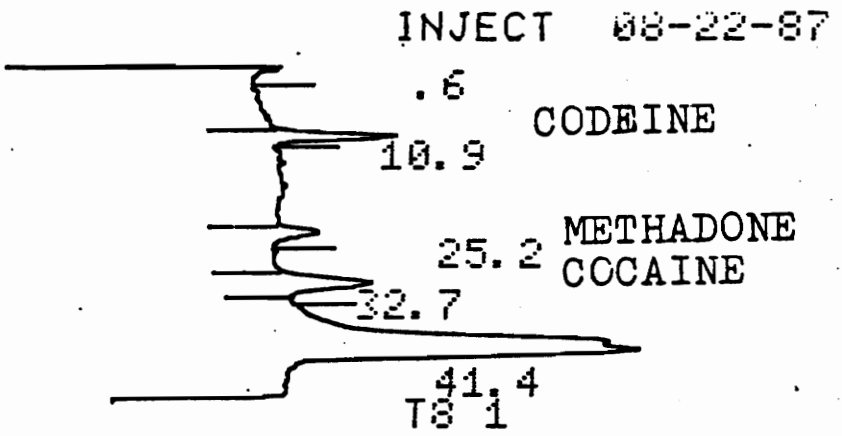


Figure 34 - E. Merck Chromatogram
(not to scale)

CONCLUSIONS

There are significant differences between plate manufacturers for both Rf and area, and the use of a single manufacturer will yield more reliable and reproducible results. The best overall results were for E. Merck's plates and Baker's plates were a very close second. The only difference was the higher signal to noise ratio of the compounds analyzed for E. Merck's plates. Analtech's plates had the most problems with both reproducibility and noise. The optimization of the solvent system for this manufacturer's plates might have made a difference.

The individual plates have a relatively uniform layer. The major surface non-uniformities can be eliminated by visual inspection of the plates by eliminating any plate with scratches, nicks or flakes. The major source of variation for all manufacturers was the plate to plate surface deviations, not the track to track deviations. The instrumentation contributed only a minor source of deviation. The plate to plate variations can be corrected with external standards (i. e., a calibration curve) analyzed on each plate.

The specific drugs analyzed in this study yielded a range of Rfs and peak areas dependent on the specific plate and manufacturer used. The results also relied heavily on the concentration of the drug with possible overloading on Analtech and Alltech plates at the higher concentrations. Alltech plates were not reliable with the concentration over 200ng and Analtech plates had resolution problems. The linearity of the curves also dropped off as the concentration of the compounds increased.

Appendix

Preliminary Studies-Spring 1987

A possible solvent system was found in the literature for the drugs of abuse chosen for this study[23]. Drugs of abuse were chosen for the wide field of interest and their general availability. The solvent system found was Ethyl acetate/Methanol/ Ammonia(aq.) (17:20:10) The Table 24 list the literature's the Rfs and the experimental Rfs this researcher obtained.

Table 24 - Results for Ethyl Acetate/Methanol/Ammonia(aq) 170:20:10

Drugs	Rf-literature	Rf-experimental
Cocaine HCl	0.67	0.85
Codeine HPO_4	0.41	0.29
Diazepam	0.64	0.88
Morphine HCl	0.23	0.21
Methadone HCl	0.65	0.63

This system was analyzed to determined its acceptability for this research. The experimental Rf's achieved differed from those listed in the reference. The experimental results were at the extremes of the desired range, 0.2-0.8, rather than contained within its limits. This lead to the testing of other solvent systems. First varying the ratios of the ethyl acetate/methanol/ammonia(aq) system were experimented. (Table 25) These systems proved to be no different or worse.

Table 25 - Rf Results for Variation of Ethyl Acetate/Alcohol/Ammonia(aq) Solvent Systems

Ratios:	30:2:1 Methanol	20:2:1 Methanol	20:1:1 Methanol	18:2:1 Methanol	17:1:1 Methanol	17:2:1 Ethanol	17:1:1 Ethanol	25:2:1 Ethanol
Cocaine	0.85	0.82	0.78	0.88	0.77	0.86	0.82	0.83
Codeine	0.19	0.27	0.19	0.30	0.19	0.21	0.17	0.17
Diazepam	0.91	0.85	0.84	0.93	0.86	0.91	0.89	0.92
Methadone	0.60	0.65	0.62	0.73	0.63	0.68	0.66	0.62
Morphine	0.12	0.16	0.10	0.21	0.11	0.15	0.11	0.11

A chloroform/ethanol system was also tried. This system separated in the development of the plate giving a secondary solvent front and tailing of the compound peaks was detected. An attempt to make this system basic, in an effort to reduce the tailing, was tried. This was thought to work since the drugs are all mildly acidic. The tailing was reduced but the separation of the solvent system increased leading to a questionable reproducibility, as well as the fact that two of the drugs did not separate from the solvent front. Other systems and some single solvents were examined and experimented on diazepam and a few were tested on morphine. (Table 26). The decision based on all the experimental results was to use the Ethyl acetate/Methanol/Ammonia(aq) in the 17:2:1 ratio first tested from the original reference. The peak shape, separation and Rfs were better than any of the other systems tested. Testing continued on all five drugs listed to verify single manufacturer and manufacturer to manufacturer variations existed and determine a need for the research. The initial testing was on E. Merck plates. The tests included testing the instrumentation as well. The tests were

1. Spotting 8 tracks - reproducibility across the plate and spotting device
2. Scanning 1 track 10 times - reproducibility of densitometer

The tests yielded the baseline variance larger than the expected and generally accepted as the industry standard of 10% (Table 27).

Table 26 - Rf Results on Other Solvent Systems

Solvent System	Rf of Diazepam	Rf of Morphine
Hexane	0	-
Toluene	0	-
Ethyl Acetate	0.75	-
Ethyl Acetate/Ammonia (17:1)	0.88	-
Methanol	0.95	-
Toluene/Ethyl Acetate/Ammonia (40:40:1)	0.64	-
Ethyl Acetate/Methanol/Ammonia (40:10:1)	0.97	-
Ethyl Acetate/Methanol/Ammonia (80:5:2)	0.92	-
Ethyl Acetate/Methanol/Ammonia (40:1:1)	0.93	-
Ethyl Acetate/Propanol/Ammonia (40:1:1)	0.91	0.05
Toluene/Ethyl Acetate/Ammonia (80:40:1)	0.4	0
Toluene/Ethyl Acetate/Ammonia (8:4:1)	0.5	0
Acetone	1	0.1
Methylene Chloride	0	0
Chloroform	1	0
Chloroform/Methanol/Ammonia (50:10:1)	1	0.4
Toluene/Chloroform/Ammonia (40:40:2)	0	0
Propanol	0.86	0.14
Acetonitrile	0.75	0.05
Toluene/Methanol/Ammonia (800:500:25)	0.8	0.36

Table 27 - Preliminary Deviation Results

	1 Spot Scanned 10 Times Area %s	8 spots Scanned 1 Time Area %s
Cocaine	11.16	16.78
Codeine	13.67	27.26
Diazepam	1.68	22.63
Methadone	24.94	11.78
Morphine	3.86	22.41

%s is percent standard deviation of mean area

The two tests were repeated on a second manufacturer, Whatman. The tests were performed with only one drug, methadone, chosen for its mid-range Rf value. Results exhibit a large deviation between the 2 manufacturers on test 1, the spotting of the multiple tracks. (Table 28)

Table 28 - Preliminary Results of Two Manufacturers Plates

Methadone Area	1 Spot / 10 Times Rf %s	1 Spot / 10 Times Area %s	9 spots / 1 Time Rf %s	9 spots / 1 Time Area %s
E. Merck plates	0.0	1.79	8.7	9.71
Whatman plates	0.0	2.55	5.8	21.89

%s is percent standard deviation of mean area

The research was decided to proceed on the basis of the preliminary results. Additional manufacturers were chosen. All available HPTLC plates were ordered from all the existing manufacturers known to this researcher. Only two manufacturers were eliminated, Magery Nagel and USI. Magery Nagel plates were unavailable, the order was place on a six month back-order. USI plates were preliminary tested to determine the Rfs and were found to totally absorb the drug mix into the layer, thereby not having any observable spots upon development, not in the solvent front nor on the initial spotting location. With this elimination, there left five manufacturers, Alltech, Analtech, Baker, E. Merck and Whatman.

It was decided to decrease the number of analytes from five to three drugs to decrease the amount of data for statistical analysis and increase separation parameters. On analyte with a low Rf, codeine, mid-range Rf, methadone, and high Rf, cocaine. Diazepam and morphine were eliminated since there Rfs were outside the desired 0.2-0.8 range.

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Vita

Maryanne V. Cleary was born on March 18, 1963, in Biddeford, Maine. After graduating with honors from Waterville High School in Waterville, Maine, she attended Colby College, receiving a Bachelor of Arts with an ACS accreditation in Chemistry in 1985. While pursuing her undergraduate studies, she worked as a summer intern at Bates College in Lewiston, Maine, with Dr. Thomas Wenzel, researching the organic synthesis of organometallic ligands for presorbent chromatographic columns.

In the Fall of 1985, she began graduate studies at VPI & SU with Dr. Thomas Hudlicky in the field or organic synthesis. She changed disciplines in the Spring of 1986, concentrating in High Performance Thin-Layer Chromatography (HPTLC) under the direction of Dr. Harold McNair. While at VPI & SU, Maryanne was a member of the American Chemical Society, graduate student secretary of the Chemistry Department Committee, lecturer and laboratory instructor for several ACS and industrial short courses involving HPTLC, GC, and HPLC, and an adjunct faculty member at New River Community College in Dublin, VA.

Maryanne V. Cleary is currently employed at Analytics Environmental Laboratory, an environmental testing firm located in Portsmouth, NH.