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Capillary Electrophoresis Used As An Alternative to HPLC For Pharmaceutical Analysis of Antifungal Agents

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ABSTRACT

CAPILLARY ELECTROPHORESIS USED AN ALTERNATIVE TO HPLC FOR PHARMACEUTICAL ANALYSIS OF ANTIFUNGAL AGENTS

HPLC is a commonly used analytical tool in the pharmaceutical industry for the characterization of drug potency and purity. However, HPLC analysis can be very time consuming, use large quantities of organics, and thus costly and not environmentally friendly. In this study, we describe an alternative to HPLC that can be used for pharmaceutical analysis. Capillary electrophoresis is being utilized increasingly for biochemistry and analytical chemistry applications. With advances in auto samplers and improvements in injection precision, the potential for this instrumentation to be used in conjunction or as an alternative to HPLC is currently being evaluated in analytical laboratories. Capillary electrophoresis allows for minimal organic consumption, fast analysis time, and high degree of resolution. In addition, capillary electrophoresis assays are more cost effective to develop and run on a routine basis due to relatively less expensive capillaries and small amounts of organic solvents. In this study, capillary electrophoresis is used for analysis of 3 classes of antifungal compounds; an imidazole, polyene and a pyrimidine, represented by miconazole, nystatin and 5-fluorocytosine. Utilizing USP validation criteria, we find comparable levels of accuracy, limit of detection (LOD), limit of quantification (LOQ), linearity, and precision to HPLC. Using, a 40 cm x 75 μm capillary with a KH_2P0_4 run buffer for both miconazole and 5-fluorocytosine, we show that both agents can be captured in less than 3 minutes with a %RSD < 2.0%, and a linearity R^2 > 0.99. For nystatin, there were solution solubility issues due to the organic: aqueous ratio and further investigation is needed to determine if comparable data to HPLC can be obtained.

INTRODUCTION

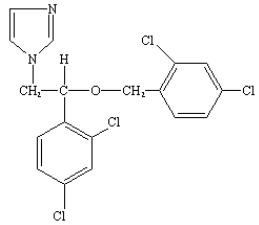
Capillary electrophoresis (CE) was developed in the early 1990s and has become an established analytical instrument in many laboratory and clinical settings with the exception of pharmaceutical analytical laboratories where HPLC has been the analytical tool of choice for the characterization of drug potency and purity. In most cases HPLC analysis is very time consuming and uses large quantities of organics and thus has higher associated method development and routine operational costs. There are many compounds for which HPLC is the optimal analytical instrument. However, there are compounds that potentially can be assayed with capillary electrophoresis and the CE's advantages of fast analysis times, smaller quantities of solutions and minimal organic usage, and thus overall lower consumable expenses can be attained. The typical amount of buffer utilized per day can be in the order of 10-100 milliliters, in comparison to HPLC which can consume liters of organic. In addition to fast analysis and cost savings, due to the inherent advantage of minimal band broadening/peak spreading, capillary electrophoresis can produce very defined peaks with a high degree of resolution [2]. A potential drawback to HPLC is the limitation of pH range to the bonded-phase materials in the columns. In comparison, CE separation is achievable in a wide pH range [7]. Injection precision has been one area that CE has been in need of improvement. The volume injected is strongly related to the viscosity of the sample [4]. Thus, internal standards have been commonly used for analysis.

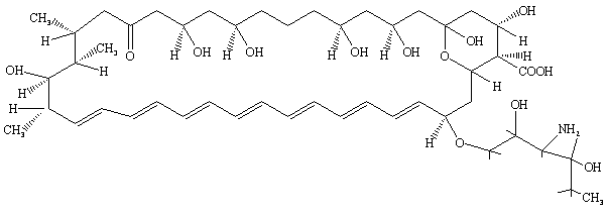
Electrophoresis has been defined as the differential movement of charged species (ions) by attraction or repulsion in an electric field [1]. Automated capillary electrophoresis separates species by applying voltage across buffer filled capillaries. It has been used more commonly for the separation of ions, which depending on size and charge, move at various speeds when voltage is applied. These solutes are seen

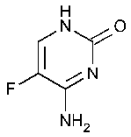
as peaks as they pass through a detector. Electrophoresis can be defined as the migration of ions under the influence of an electric field, $F_{E,}$ which is proportional to its effective charge, q, and the electric field strength, E. ($F_E = qE$) [8].

The main separation modes used in capillary electrophoresis include capillary zone electrophoresis, micellar electrokinetic capillary chromatography, capillary isotachophoresis, capillary gel electrophoresis, and capillary isoelectric focusing. For the purpose of this study, capillary zone electrophoresis (CZE) will be employed. In this mode, the sample is applied as a narrow zone (band), which is surrounded by the separation buffer. As the electric field is applied, each component in the sample zone migrates according to its own apparent mobility. It is intended that all sample components will eventually separate from each other to form individual zones of pure material. In practice, neutral molecules cannot be separated due to the fact that they migrate at the velocity of the electroosmotic flow. The separation of charged molecules is accomplished most effectively when the differences among the velocities of the solutes are maximized and random dispersion of the zones are minimized. Cations migrate through the capillary in the same direction as electroosomitic flow, from the anode to the cathode. Cations also migrate at a faster rate than the electroosomitc flow. They elute in order of their charge-to-size ratios, thus the small, highly charged cations elute first. As noted, neutral molecules are not separated from each other, but move through the capillary under the influence of only the electroosmotic flow. Anions elute in reverse order to their charge-to-size ratios with the small, highly charged anions eluting last. Thus, the overall elution order will be small highly charged cations, neutral molecules, and small highly charged anions eluting last [8].

For the purpose of this investigation three classes of antifungals will be examined with capillary electrophoresis. An imidazole, a polyene, and a pyrimidine, represented by miconazole, nystatin, and 5-fluorocytosine respectively.







Miconazole **Nystatin** **5-fluorocytosine**

The molecular weight for these compounds are 416.12, 926.11, and 129.09 for miconazole, nystatin, and 5-fluorocytosine respectively. The USP criteria that will be evaluated in this study are linearity, precision, quantitation limit (LOQ), and detection limit (LOD). The linearity of an analytical procedure is the ability to yield test results that are directly proportional to the concentration of the analyte in the samples within a given range [3]. Precision refers to the degree of agreement among injections of the same solution [3]. The quantitation limit refers to the lowest amount of analyte that can be quantified. The detection limit is the lowest amount of analyte that can be detected, but not necessarily quantified [3].

Other similar studies have shown capillary electrophoresis as being comparable to HPLC. In one such study, CE was utilized in a pharmaceutical quality control laboratory. Typical drug CE assays were found to have 1% RSD, with linearity values of R^2=0.999. Other factors, such as method robustness, sensitivity, accuracy, and reagent stability were comparable to HPLC [4].

In a similar study, CE was used for the quantification of mirtazapine and related impurities. The %RSD standard values were found to be between 2-3%, which required the use of an internal standard. However, other validated method criteria were found to be comparable to HPLC [6].

In yet another study, a validated method utilizing single borate buffer allowed for analysis of a wide range of acidic compounds, including active drugs, excipients, starting materials, and intermediates. The method allowed for acceptable injection precision with use of an internal standard. The cost and time savings in comparison to HPLC were found to be significant [5].

MATERIAL AND METHODS (EXPERIMENTAL DESIGN)

Three separate methods were developed, and subsequent experiments were performed on a Beckman Coulter Capillary electrophoresis system with a Photo-diode-array (PDA) detector. The system was run with Beckman Coulter 32 Karat v8.0 software. Data was collected with Waters Empower data collection system. A fused-silica capillary at 40cm in length to the detector window and an internal diameter of 75µm was used for all tests.

The hydrodynamic injection volume for 5-fluorocytosine was 43nL, which corresponds to a sample introduction at 0.4psi for 9 seconds. The hydrodynamic injection volume for miconazole was 115nL, which corresponds to a sample introduction at 0.8psi for 12 seconds. The hydrodynamic injection volume for nystatin was 14nL, which corresponds to a sample introduction at 0.3psi for 4.0 seconds. For detection, the PDA detector was at 214nm for all three compounds. Voltage used for analysis were 25kV, 20kV, and 30kV for 5-fluorocytosine, miconazole, and nystatin respectively. The capillary temperature was set to 25°C. A 10 mM potassium phosphate (monobasic) buffer (KH_2PO_4), pH7.0 was used for both 5-fluorcystosine and Nystatin. A 50mM KH_2P0_4 was utilized for miconazole. Data analysis time was 4 minutes for 5-fluorocytosine, 7 minutes for miconazole, and 3 minutes for nystatin.

Table 1. Antifungal CE Method Instrument Parameters

Antifungal Agents	Analysis Time	Buffer	Injection Volume (nL)	Temperature (°C)	Voltage (kV)
5-fluorocytosine	4 minutes	10mM KH_2PO_4, pH 7.0	43	25.0	25
Miconazole	7 minutes	50mM KH_2PO_4, pH 4.5	115	25.0	20*
Nystatin	3 minutes	10mM KH_2PO_4, pH 7.0	14	25.0	30

* (reverse polarity applied)

Table 2. REAGENTS

Reagent
Potassium phosphate monobasic (KH_2PO_4)
50% Sodium Hydroxide
Methanol
Millipore Water
Dimethylformamide

BUFFER SOLUTIONS

Potassium phosphate monobasic (KH_2PO_4) at working concentrations of 10mM and 50mM were weighed out on a Mettler-Toledo analytical balance. For miconazole, 3.45g of KH_2PO_4 was transferred to 500 ml of Millipore water. For 5-fluorocytosine and nystatin 1.36g of KH_2PO_4 was transferred to 1 liter of Millipore water. The pH was adjusted for the 10mM concentration to 7.0 using 50% sodium hydroxide. All solutions were vacuum/degassed for about 10 minutes and filtered through a 0.2µm (PALL) nylon membrane filter before loading into the autosampler.

REFERENCE SOLUTIONS

Table 3. Reference Standards

Standard
5-fluorocytosine
Miconazole
Nystatin

Reference stock solutions were prepared at initial concentrations of 2.0mg/ml for all 3 antifungal agents. Miconazole was dissolved in 100% methanol. 5-fluorocytosine was dissolved into the 10mM KH_2PO_4, pH7.0 buffer. Nystatin was dissolved into a 50:50 solution of dimethylformamide and KH_2PO_4, pH7.0 buffer.
For the linearity study, a concentration range of 0.7mg/ml to 1.3 mg/ml was prepared for both miconzole and 5-fluorocytosine. A reference point (100%) at 1.0mg/ml was established, and thus the overall range represented 70% to 130%.

Due to the higher molecular weight of nystatin, a lower target reference concentration of 0.5mg/ml was selected as the reference 100%. The linearity solutions ranged from 0.00025mg/ml to 1.1 mg/ml. For nystatin, the 0.00025mg/ml solution represented the Limit of Quantification (LOQ) concentration, which is 0.05% of the target concentration of 0.5mg/ml. The overall linearity range evaluated was 0.05% to 220% of target of 0.5mg/ml.

An additional check solution was prepared for each compound at the target concentration of 0.5mg/ml for nystatin and 1.0mg/ml for 5-fluorocytosine and miconazole.

All above outlined working standard solutions were dissolved into their respective buffers. For miconazole solutions, 50mM KH_2P0_4, pH 4.5 buffer and for 5-fluorocytosine and nystatin, 10mM kH_2P0_4, pH 7.0 buffer. All solutions were filtered through a 0.2µm nylon membrane filter before loading into the autosampler.

A limit of detection (LOD) and limit of quantification (LOQ) study was prepared for 5-fluorocytosine since this was the smallest molecule of the group and thus was used to challenge the CE system. A concentration of 0.0005mg/ml, representing 0.05% of the 1mg/ml target concentration was prepared.

COLUMN REGERNATION/WASH SOLUTION

After every 5 or 7 seven-sample injections, the capillary was washed with 0.1M sodium hydroxide at 20psi for 1 minute.

RESULTS AND DISCUSSION

Influence of Buffer Concentration and Voltage

In capillary electrophoresis, in addition to solutes, the run buffer also moves through the capillary under the influence of an electric field [8]. This phenomenon is referred to as electroosmotic flow [8].

The ionic strength of KH_2P0_4 was examined to determine effect of peak elution and peak symmetry. If temperature is controlled, an increase in the buffer ionic strength will reduce the electroosmotic flow. However, if temperature is not controlled, an increase in buffer ionic strength will increase the osmotic flow due to and increase in current and temperature which will lower viscosity [9]. In general although lower buffer concentrations should produce faster analysis times; concentrations that are excessively low may produce broadened and asymmetric peak shapes [9]. For this study, the capillary temperature was set to a 25C. Considering only one analyte was needed to be separated per analysis, the findings with regard to voltage and buffer concentration effected elution time and peak shape mainly. With one analyte

involved there was considerable flexibility in choosing a final buffer/voltage combination.

For all conditions, the capillary temperature was set to 25°C. Miconazole was examined with both 50mM pH 4.5, and 100mM, pH 4.5 kH_2P0_4 run buffer. There was a slight increase in retention time for Miconazole with the 100mM run buffer if the voltage was the same. The differences between the buffer concentrations were less than 1 minute. With a higher ionic strength run buffer, higher current levels were generated with the same voltage. Although, initially there did not seem to be any negative influence, a concern with joule heating was considered when higher currents were generated. With the longer analysis times needed for the linearity study, this was a reason for concern. Thus, a balance of the run buffer ionic strength and voltage was needed to provide quick elution time, without generating too much system current and thus heating. A 50mM KH_2P0_4, pH 4.5 with 20KV was decided upon for the miconazole assay. The 50mM KH_2P0_4 provided for a peak elution time of miconazole at about 3 minutes and less current generation.

As discussed, different voltages ranging from 10kV to 30kV were also examined in conjunction with buffer concentrations. Considering the electric field, E, is the voltage/length, changing the voltage allows for a modification of the electoosmotic flow due to the variation in the electric field [8]. An increase in voltage will increase the electroosmotic flow and reduce analyte retention times. The utilization of higher voltages will provide higher efficiencies. However, high voltages will also produce increased joule heating due to the production of higher current.
With regard to 5-fluorocytosine and nystatin, it was decided to evaluate a 10mM KH_2P0_4 run buffer. The benefit of this concentration allowed for more flexibility in terms of selecting voltages. The lower buffer concentration produced less current and therefore less heat with a given voltage setting.

For 5-fluorocytosine, a 10mM KH_2P0_4 run buffer with 25kV of voltage was utilized. A voltage setting of 30kV was used with the 10mM KH_2P0_4 run buffer for nystatin.

Influence of pH

The run buffer pH has a significant effect on electroosomotic flow because it changes the zeta potential, which is proportional to the surface charge on the capillary wall. As the pH of the run buffer increases, there is an increase in the electroosmotic flow, due to the fact that there is more dissociation of the Si-OH to $Si\text{-}O^-$ on the inner capillary wall. In addition, at higher pH there are more charged $Si\text{-}O^-$ groups and, thus a greater zeta potential leading to an increase in electroosmotic velocity. Conversely, at lower pH levels there is less surface ionization and a lower zeta potential [8]. The run buffer pH will also have impact on the degree of ionization of the solutes and thus their mobility's [8].

pH can potentially play a greater role than buffer concentration and voltage in the final result of an assay. For this investigation, with only one analyte to evaluate, differences in buffer pH did not significantly influence the chromatography. Thus, there was more flexibility in determining a suitable pH. For an analysis with multiple analytes to separate, the pH of the buffer would play a large role. It is likely that slight changes would influence the separation characteristics significantly.

In general it is recommended to select a run buffer at pH around or slightly lower than the pKa value of the compound. Miconazole and 5-fluorocytosine have pKa values of 6.7 and 3.2 respectively. However, initially run buffers of pH 3.0 and 4.5 were prepared at both the 50mM and 100mM concentrations for miconazole. At each buffer concentration, pH 4.5 yielded a slightly quicker elution time.

For 5-fluorocytosine, 10mM KH_2P0_4 buffer was prepared at pH 11.0 and 7.0 It was found that the peak shape was more symmetrical with the buffer at pH 7.0. In addition analyte elution time was quicker. The finding with nystatin was similar; with peak shape significantly better at pH 7.0 than at pH 11.0.

Additional investigations with regard to pH possibly would have yielded even better chromatography (in terms of peak shape), particularly with miconazole. However, the validation criteria of this study were sufficiently met with the selected buffer pH.

Influence of Temperature

An increase in temperature can cause an increase in electroosmotic flow due to a decrease in the viscosity of the buffer [8]. A 1°C temperature increase will cause a 2.4% decrease in the viscosity of water [8]. An increase in temperature also will cause a decrease in the dielectric constant, which will decrease the electroosmotic

flow [8]. Thus, it would seem that these two factors would cancel each other. However, the decrease in dielectric constant for water is minimal. Thus, the change in viscosity would have the greatest effect on electroosmotic flow [8]. As noted in the buffer molarity and voltage discussion, maintaining a set temperature is essential to avoid thermal heating. For this study, the temperature was set to 25°C. No additional experiments were performed to evaluate the effect of temperature changes.

Influence of organic solvent

Due to the solubility properties of miconazole and nystatin, organic solvents were needed. Miconazole is soluble in methanol, and nystatin is soluble in dimethyformamide. Miconazole was dissolved in 100% methanol for the stock (initial) standard solution. For nystatin, the stock standard solution was dissolved in a 50:50 dimethylformamide and 10mM KH2P04, pH 7.0 buffer. This ratio of (50:50 aqueous:organic) caused solution solubility issues that were apparent when reviewing the results of the linearity study as discussed later in the findings. In addition, although the precision study yielded replicate injections of less than 2.0%, it is believed that the solution stability of nystatin also adversely affected precision.

The effect of organic solvent can be variable due to several factors including viscosity, dielectric constant, and zeta potential [8]. However, for the purpose of meeting the validation criteria of linearity and precision considered in this study, it was necessary to minimize as much a reasonable the amount of organic added to the final antifungal working standards.

This was not an issue for 5-fluorocytosine due to its solubility in water. For miconaolze and nystatin, a balance was needed to be achieved between the levels of organic and water. If the organic level was too high in the final working standard solutions, precision would be adversely affected. In addition, current generation could be inhibited if the organic level was too high in the sample.

Initially miconazole was prepared for analysis in a (25:75 water:methanol) solution. Relative standard deviation (%RSD) was typically found at around 5% - 7%. New solutions were prepared at a ratio of (50:50 methanol and 50mM KH_2P0_4, pH 4.5) run buffer. This resulted in better sample precision with values of less than 2.0% RSD for all concentration levels.

The findings were similar for nystatin and the use of dimethlyformamide to dissolve the compound. The final working solutions for nystatin were at (25:75 organic to KH2PO4, pH 7.0) run buffer. Although injection precision was less than 2% RSD; higher values were found in comparison to miconazole and 5-fluorocytosine (see table). However, the results of linearity study with R^2 values at 0.89 were not acceptable. It was first considered that the issue was due to not allowing enough time for cooling the mixture of dimethylformamide and buffer, when preparing the final working solutions. In a second preparation of the working standard solutions, a considerably longer amount of time was allowed for more than adequate solution cooling. The results of this analysis (data not shown) were the same as the first analysis. Upon visual inspection of the flasks, there did not appear to be any particulate formation. However, since precision results were less than 2.0% across the entire concentration range, it is believed that the level of buffer in the working solutions was too high and nystatin was not stable in the solution. Further investigation was not performed, but this can potentially cause problems with molecules that have solubility issues in an partially aqueous environment.

Influence of buffer depletion and vial levels

The influence of run buffer depletion was evaluated during this study. A number of factors are involved before run buffer “depletion” and loss of effectiveness. Changes in buffer pH or composition can cause changes in electroosmotic flow, which will result in changes in migration times and peak areas [8].

Another consideration to buffer depletion is change in the composition of the solution itself. Buffer pH in the source and destination vials can change. This can occur due to electrolysis of water, where protons are produced at the cathode and hydroxide ions at the anode [8]. In addition, changes in buffer can occur due to the electrolysis and migration of buffer ions. Finally, buffer composition can change in the destination vial if solute ions exit the capillary. If there are sufficient quantities of solutes present in the destination vial, a change in the electric field could occur which in turn could change the electric field strength in the capillary.
With regard to nystatin, it could also be possible that nystatin stability changed the composition of the destination vial.

Overall, the number of injections that can be made between replenishment with new buffer is dependent on the magnitude and duration of the current flow, the volumes and quantities of the sample injected, and the buffer's capacity [8].

In this study, there did not appear to be a set consistent number of injections that could be performed before adverse affects in chromatography were observed. However, for both the 10mM and 50mM KH_2PO_4 buffers used, it was apparent that at least 10 samples could be analyzed before the possibility of adverse effects.

Another factor to be considered is source and destination run buffer vial levels. It is critical the level of these vials be even. If for example the level of the source vial is higher than the destination vial, siphoning will occur and thus introduce laminar flow [8]. The result would be shorter peak elution time. Conversely, if the level of the destination vial was higher than the source vial, migration times would be longer. Since migration time changes would occur, peak area could also change as a result.

Influence of capillary conditioning and regenerating

During method development for all 3 antifungal agents, the effect of both capillary conditioning and regenerating was evaluated. It was determined early on that an injection of the run buffer with voltage would be applied before the sample injection. The electroosmotic flow and migration times are very sensitive to conditions of the capillary surface. Preconditioning the capillary influences migration times [8]. The equilibration injection marginally improved the %RSD between multiple injections. It also provided an additional benefit as a column wash to ensure there would be no carry-over. In addition, peak retention times were found to be consistent within each analysis.

In addition to an equilibration injection before introduction of the sample, the capillary was regenerated after every 5-12 injections with 0.1M sodium hydroxide. Washing the capillary with a basic solution regenerates the silica surface by removing any solutes or buffer ions from the inner wall [8]. Since the silica is soluble in basic solution, some silica is dissolved and new silica is exposed. Washing a capillary with 0.1M sodium hydroxide is beneficial if the migration times are initially unstable [8].

During the development of the miconazole and 5-fluorocytosine methods, the capillary was regenerated with 0.1M sodium hydroxide after every 10-12 injections. With the nystatin analysis, the capillary was regenerated after every 5-7 injections.

Influence of sample introduction and time

Unlike HPLC where injection volume is selected; for capillary electrophoresis sample introduction is achieved by pressure and time for hydrodynamic injections used in this study. The Hydrodynamic injection mode was selected since it is believed to be more precise and robust than electrokinetic injection [10]. The injection conditions are generally only affected if the viscosity of the buffer is drastically changed by temperature. Similar to other method development parameters for this instrument, there does not seem to be a rule or setting that will produce the best results. Instead, it is a matter of optimization for the particular compound and buffer. However, longer, lower pressure injection gives the instrumentation more time to respond to variances and will potentially provide a better result [11]. After the pressure was selected, the longest reasonable injection time was allotted without increasing the sample injection volume significantly. In this study, no significant findings were apparent from varying injection pressure and time. Thus, parameters were selected based on molecule size. In the case of miconazole, however, a greater injection volume was selected relative to the smaller 5-fluorocytosine, to achieve good precision.

Linearity, Precision, LOQ, and LOD Results

The focus of this study has been to achieve results similar to HPLC for linearity and precision. Although the system was challenged with only one analyte, comparable findings are shown with miconazole and 5-fluorocytosine. Refer to the data tables to obtain all the results related to these studies.

Overall for miconazole, standard agreement at 5 injections was found to be less than 2.0% for all concentration levels. The results of the linearity study show R^2 values at 0.996 (study 1) and R^2 values at 0.994 (study 2).

The results are similar for 5-fluorocytosine, with precision values of less than 2.0% and R^2 values at 0.998 for the linearity study. A limit of quantification (LOQ) result of signal to noise ratio of 11, with a 3.4% RSD, was achieved at a concentration of 0.0025mg/ml. A limit of detection (LOD) result with signal to noise ratio of 4, 26.0% RSD, was found at a concentration of 0.0005mg/ml.

Nystatin proved to be more challenging to work with than miconazole and 5-fluorocytosine. With further investigation, the results for this compound may be very similar. With regard to precision, less than 2.0% RSD was achieved throughout each concentration level. As discussed previously, due to solution solubility issues, the results of linearity were not acceptable as per USP validation criteria.

Analytical Data for Miconazole

Table 4. (Miconazole – check standard analysis MCZ_std_validation_061808)

Sample Name	Injection number	Result ID	Retention Time	Area
Miconazole – 1.0mg/ml	1	3389	2.75	804550
Miconazole – 1.0mg/ml	2	3390	2.75	810536
Miconazole – 1.0mg/ml	3	3391	2.76	813068
Miconazole – 1.0mg/ml	4	3392	2.75	812577
Miconazole – 1.0mg/ml	5	3393	2.76	817372
Mean				811621
%RSD				0.6

Sample Name	Injection number	Result ID	Retention Time	Area
Miconazole – 1.0mg/ml (check)	1	3394	2.75	813855
Miconazole – 1.0mg/ml (check)	2	3395	2.75	821108
Mean				817482
%RSD				0.6

Average areas used for calculations
Miconazole standard weight = 500.48mg/250ml volumetric flask
Miconazole check standard weight = 100.56mg/50ml volumetric flask
Standard Agreement = 99.7%

Sample Set Name: CNL_MCZ_Lin_study1_061808

Table 5. (Miconazole Linearity Study)

Sample Name	Injection number	Retention Time	Area
Miconazole - 0.7 mg/ml	1	2.79	564064
Miconazole - 0.7 mg/ml	2	2.80	577168
Miconazole - 0.7 mg/ml	3	2.78	564049
Miconazole - 0.7 mg/ml	4	2.76	581151
Miconazole - 0.7 mg/ml	5	2.75	581389
Mean			573564
% RSD			1.5

Table 6. (Miconazole Linearity Study 1)

Sample Name	Injection number	Retention Time	Area
Miconazole - 0.8 mg/ml	1	2.73	653576
Miconazole - 0.8 mg/ml	2	2.72	668840
Miconazole - 0.8 mg/ml	3	2.73	683716
Miconazole - 0.8 mg/ml	4	2.73	675764
Miconazole - 0.8 mg/ml	5	2.73	673544
Mean			671088
% RSD			1.7

Table 7. (Miconazole Linearity study 1)

Sample Name	Injection number	Retention Time	Area
Miconazole - 1.0 mg/ml	1	2.75	819261
Miconazole - 1.0 mg/ml	2	2.75	838945
Miconazole - 1.0 mg/ml	3	2.75	830247
Miconazole - 1.0 mg/ml	4	2.75	824237
Miconazole - 1.0 mg/ml	5	2.75	818514
Mean			826241
%RSD			1.0

Figure 1. (miconazole 1.0mg/ml concentration)

Chromatogram Report_mcz

Sample Set Name: CNL_MCZ_Lin_study2_062108	Acquired By: CLechnou
Project Name: Titusville NJ\Training\C. Lechnou Reseach	Date Printed: Tuesday, July 15, 200
Result Set Id: 4140	Calibration Id: 4141

Acq Method Set: CE_MS	System Name: TI_CE_Beckman
Date Acquired: Saturday, June 21, 2008 4:21:18 PM EDT	Channel Description:
Date Processed: Saturday, June 21, 2008 9:53:36 PM EDT	Channel Id: 3784
Processing Method: CNL_lin2	Result Id: 4149
SampleName: MCZ-1.0 mg/ml	Vial: 3 Injection: 2

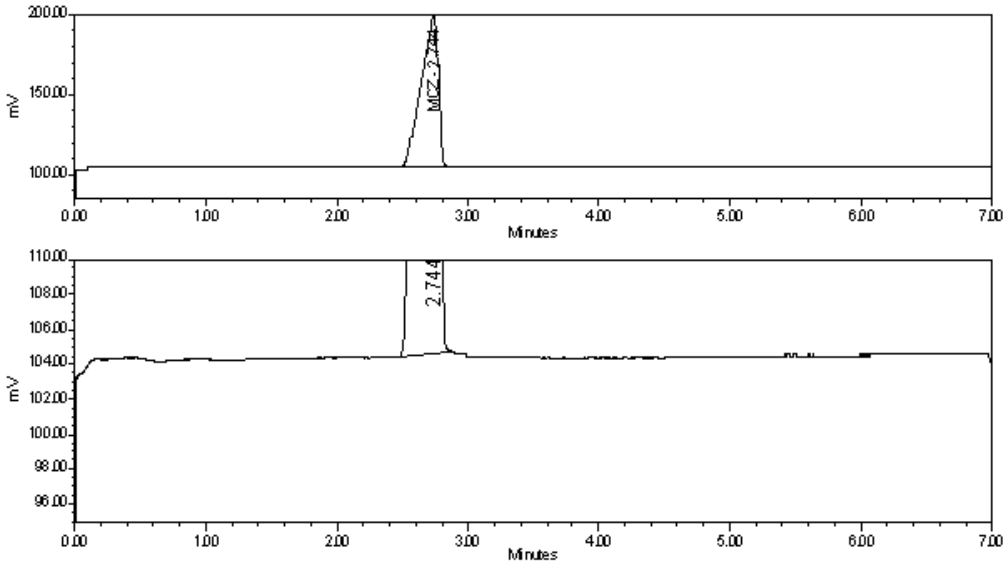


Figure 2. Linearity study 1
Miconazole

Linearity Standard Report_CNL

Sample Set Name: CNL_MCZ_Lin_study1_061808
Project Name: Titusville NJ\Training\C. Lechnou Reseach
Result Set Id: 3723

Acquired By: CLechnou
Calibration Id: 3724

Component Results

	Equation	Name	RT	Area (µV*sec)	R^2	Concentration	R
1	Y = 8.33e+005 X - 4.27e+003	MCZ	2.794	564064	0.996417	0.701	0.998207
2	Y = 8.33e+005 X - 4.27e+003	MCZ	2.796	577168	0.996417	0.701	0.998207
3	Y = 8.33e+005 X - 4.27e+003	MCZ	2.782	564049	0.996417	0.701	0.998207
4	Y = 8.33e+005 X - 4.27e+003	MCZ	2.760	581151	0.996417	0.701	0.998207
5	Y = 8.33e+005 X - 4.27e+003	MCZ	2.750	581389	0.996417	0.701	0.998207
6	Y = 8.33e+005 X - 4.27e+003	MCZ	2.726	653576	0.996417	0.801	0.998207
7	Y = 8.33e+005 X - 4.27e+003	MCZ	2.722	668840	0.996417	0.801	0.998207
8	Y = 8.33e+005 X - 4.27e+003	MCZ	2.730	683716	0.996417	0.801	0.998207
9	Y = 8.33e+005 X - 4.27e+003	MCZ	2.730	675764	0.996417	0.801	0.998207
10	Y = 8.33e+005 X - 4.27e+003	MCZ	2.725	673544	0.996417	0.801	0.998207
11	Y = 8.33e+005 X - 4.27e+003	MCZ	2.747	819261	0.996417	1.001	0.998207
12	Y = 8.33e+005 X - 4.27e+003	MCZ	2.748	838945	0.996417	1.001	0.998207
13	Y = 8.33e+005 X - 4.27e+003	MCZ	2.750	830247	0.996417	1.001	0.998207
14	Y = 8.33e+005 X - 4.27e+003	MCZ	2.750	824237	0.996417	1.001	0.998207
15	Y = 8.33e+005 X - 4.27e+003	MCZ	2.747	818514	0.996417	1.001	0.998207

Component Results

	Residual Sum of Squares	Equation
1	1.163628e+008	Y = 8.33e+005 X - 4.27e+003
2	1.163628e+008	Y = 8.33e+005 X - 4.27e+003
3	1.163628e+008	Y = 8.33e+005 X - 4.27e+003
4	1.163628e+008	Y = 8.33e+005 X - 4.27e+003
5	1.163628e+008	Y = 8.33e+005 X - 4.27e+003
6	1.163628e+008	Y = 8.33e+005 X - 4.27e+003

Figure 3a. Miconazole
Linearity plot - ave. points

Linearity Standard Report_MCZ

Sample Set Name: CNL_MCZ_Lin_study1_061808	Acquired By: CLechnou
Project Name: Titusville NJ\Training\C. Lechnou Reseach	Date Printed: Monday, July 14, 200
Result Set Id: 3723	Calibration Id: 3724

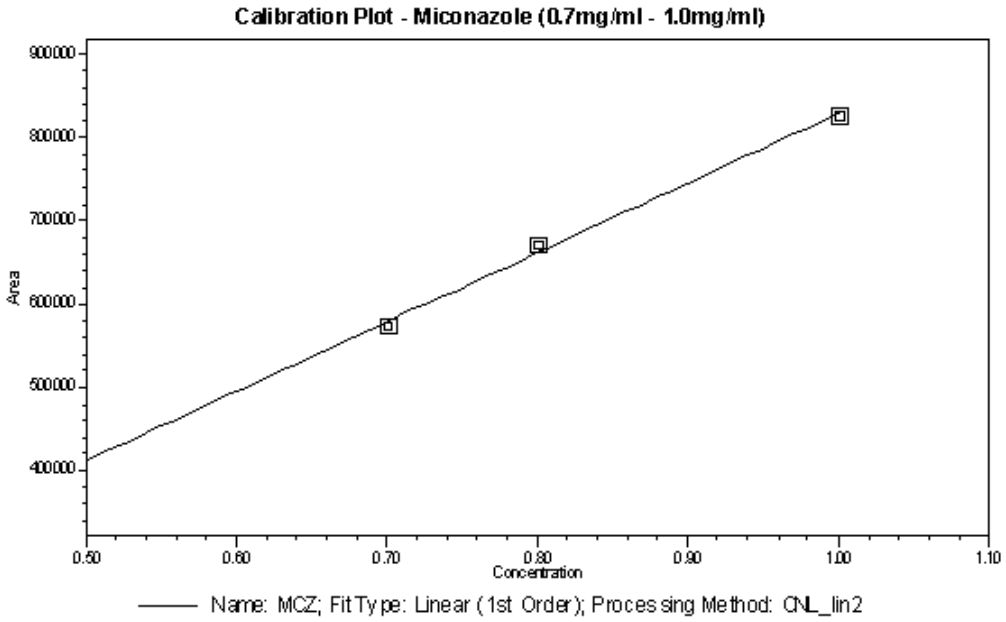


Figure 3b. All points

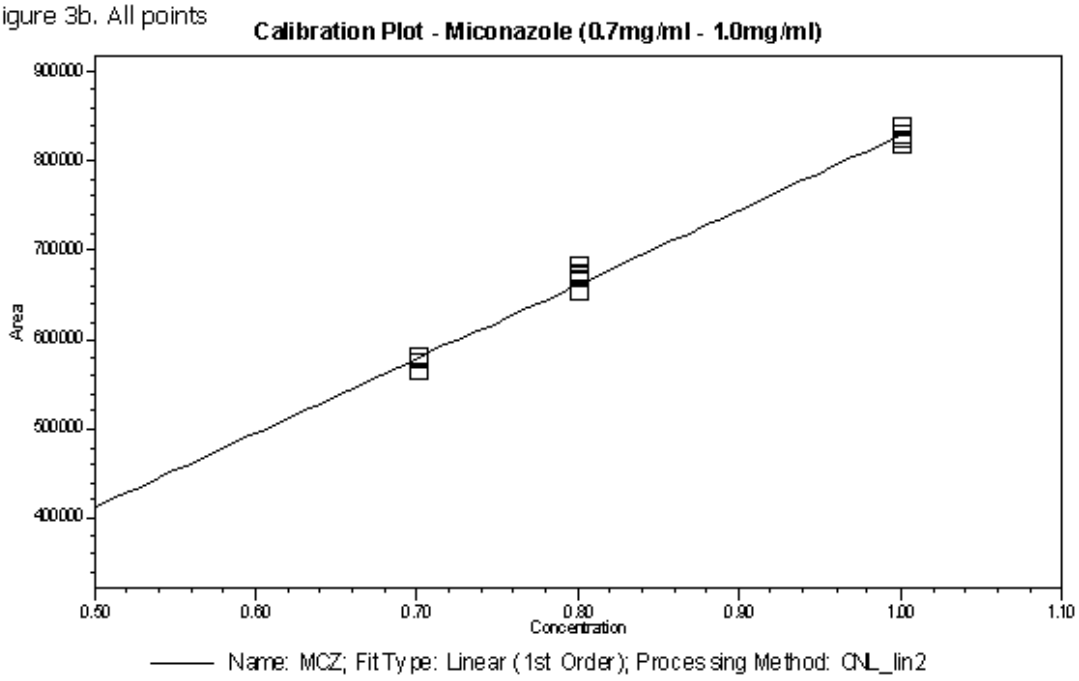


Table 8. (Miconazole Linearity Study 2- CNL_MCZ_Lin_study1_062108)

Sample Name	Injection number	Result ID	Retention Time	Area
Miconazole - 0.9 mg/ml	1	4143	2.76	732972
Miconazole - 0.9 mg/ml	2	4144	2.74	709822
Miconazole - 0.9 mg/ml	3	4145	2.73	739711
Miconazole - 0.9 mg/ml	4	4146	2.73	735856
Miconazole - 0.9 mg/ml	5	4147	2.72	742759
Mean				732224
% RSD				1.8

Table 9. (Miconazole Linearity Study 2)

Sample Name	Injection number	Result ID	Retention Time	Area
Miconazole - 1.0 mg/ml	1	4148	2.74	841296
Miconazole - 1.0 mg/ml	2	4149	2.74	854341
Miconazole - 1.0 mg/ml	3	4150	2.74	855083
Miconazole - 1.0 mg/ml	4	4151	2.74	845032
Miconazole - 1.0 mg/ml	5	4152	2.74	848947
Mean				848940
% RSD				0.7

Table 10. (Miconazole Linearity Study 2)

Sample Name	Injection number	Result ID	Retention Time	Area
Miconazole - 1.1 mg/ml	1	4153	2.79	930245
Miconazole - 1.1 mg/ml	2	4154	2.79	930178
Miconazole - 1.1 mg/ml	3	4155	2.77	924661
Miconazole - 1.1 mg/ml	4	4156	2.77	924809
Miconazole - 1.1 mg/ml	5	4157	2.76	927187
Mean				927416
%RSD				0.3

Table 11. (Miconazole Linearity Study 2)

Sample Name	Injection number	Result ID	Retention Time	Area
Miconazole - 1.2 mg/ml	1	4158	2.78	990378
Miconazole - 1.2 mg/ml	2	4159	2.77	1006883
Miconazole - 1.2 mg/ml	3	4160	2.77	1001676
Miconazole - 1.2 mg/ml	4	4161	2.76	1004755
Miconazole - 1.2 mg/ml	5	4162	2.77	1014703
Mean				1003679
%RSD				0.9

Table 12. (Miconazole Linearity study 2)

Sample Name	Injection number	Result ID	Retention Time	Area
Miconazole - 1.3 mg/ml	1	4163	2.83	1074690
Miconazole - 1.3 mg/ml	2	4164	2.83	1097089
Miconazole - 1.3 mg/ml	3	4165	2.83	1104802
Miconazole - 1.3 mg/ml	4	4166	2.82	1100162
Miconazole - 1.3 mg/ml	5	4167	2.82	1102949
Mean				1095939
%RSD				1.1

Figure 4a. Miconazole Linearity Study 2

Linearity Standard Report_CNL

Sample Set Name: CNL_MCZ_Lin_study2_062108
Acquired By: CLechnou
Project Name: Titusville NJ\Training\C. Lechnou Reseach
Date Printed: Monday, July 14, 200
Result Set Id: 4140
Calibration Id: 4141

Component Results

	Equation	Name	RT	Area (µV*sec)	R^2	Concentration	R
1	Y = 8.83e+005 X - 5.02e+004	MCZ	2.755	732972	0.994060	0.900	0.997025
2	Y = 8.83e+005 X - 5.02e+004	MCZ	2.739	709822	0.994060	0.900	0.997025
3	Y = 8.83e+005 X - 5.02e+004	MCZ	2.735	739711	0.994060	0.900	0.997025
4	Y = 8.83e+005 X - 5.02e+004	MCZ	2.726	735856	0.994060	0.900	0.997025
5	Y = 8.83e+005 X - 5.02e+004	MCZ	2.721	742759	0.994060	0.900	0.997025
6	Y = 8.83e+005 X - 5.02e+004	MCZ	2.745	841296	0.994060	1.001	0.997025
7	Y = 8.83e+005 X - 5.02e+004	MCZ	2.744	854341	0.994060	1.001	0.997025
8	Y = 8.83e+005 X - 5.02e+004	MCZ	2.737	855083	0.994060	1.001	0.997025
9	Y = 8.83e+005 X - 5.02e+004	MCZ	2.744	845032	0.994060	1.001	0.997025
10	Y = 8.83e+005 X - 5.02e+004	MCZ	2.736	848947	0.994060	1.001	0.997025
11	Y = 8.83e+005 X - 5.02e+004	MCZ	2.786	930245	0.994060	1.100	0.997025
12	Y = 8.83e+005 X - 5.02e+004	MCZ	2.786	930178	0.994060	1.100	0.997025
13	Y = 8.83e+005 X - 5.02e+004	MCZ	2.770	924661	0.994060	1.100	0.997025
14	Y = 8.83e+005 X - 5.02e+004	MCZ	2.769	924809	0.994060	1.100	0.997025
15	Y = 8.83e+005 X - 5.02e+004	MCZ	2.764	927187	0.994060	1.100	0.997025
16	Y = 8.83e+005 X - 5.02e+004	MCZ	2.778	990378	0.994060	1.200	0.997025
17	Y = 8.83e+005 X - 5.02e+004	MCZ	2.775	1006883	0.994060	1.200	0.997025
18	Y = 8.83e+005 X - 5.02e+004	MCZ	2.766	1001676	0.994060	1.200	0.997025
19	Y = 8.83e+005 X - 5.02e+004	MCZ	2.765	1004755	0.994060	1.200	0.997025
20	Y = 8.83e+005 X - 5.02e+004	MCZ	2.769	1014703	0.994060	1.200	0.997025
21	Y = 8.83e+005 X - 5.02e+004	MCZ	2.832	1074690	0.994060	1.300	0.997025
22	Y = 8.83e+005 X - 5.02e+004	MCZ	2.825	1097089	0.994060	1.300	0.997025
23	Y = 8.83e+005 X - 5.02e+004	MCZ	2.828	1104802	0.994060	1.300	0.997025
24	Y = 8.83e+005 X - 5.02e+004	MCZ	2.821	1100162	0.994060	1.300	0.997025
25	Y = 8.83e+005 X - 5.02e+004	MCZ	2.819	1102949	0.994060	1.300	0.997025

Figure 4b. Miconazole Linearity
Study 2

Linearity Standard Report_CNL

Sample Set Name: CNL_MCZ_Lin_study2_062108
Project Name: Titusville NJ\Training\C. Lechnou Reseach
Result Set Id: 4140

Acquired By: CLechnou
Date Printed: Monday, July 14, 200
Calibration Id: 4141

Component Results

	Residual Sum of Squares	Equation
1	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
2	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
3	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
4	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
5	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
6	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
7	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
8	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
9	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
10	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
11	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
12	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
13	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
14	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
15	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
16	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
17	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
18	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
19	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
20	4.651646e+008	Y = 8.83e+005 X - 5.02e+004

Component Results

	Residual Sum of Squares	Equation
21	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
22	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
23	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
24	4.651646e+008	Y = 8.83e+005 X - 5.02e+004
25	4.651646e+008	Y = 8.83e+005 X - 5.02e+004

Figure 5a. Miconazole
Linearity plot-average points

Linearity Standard Report_CNL

Sample Set Name: CNL_MCZ_Lin_study2_062108	Acquired By: CLechnou
Project Name: Titusville NJ\Training\C. Lechnou Reseach	Date Printed: Monday, July 14, 200
Result Set Id: 4140	Calibration Id: 4141

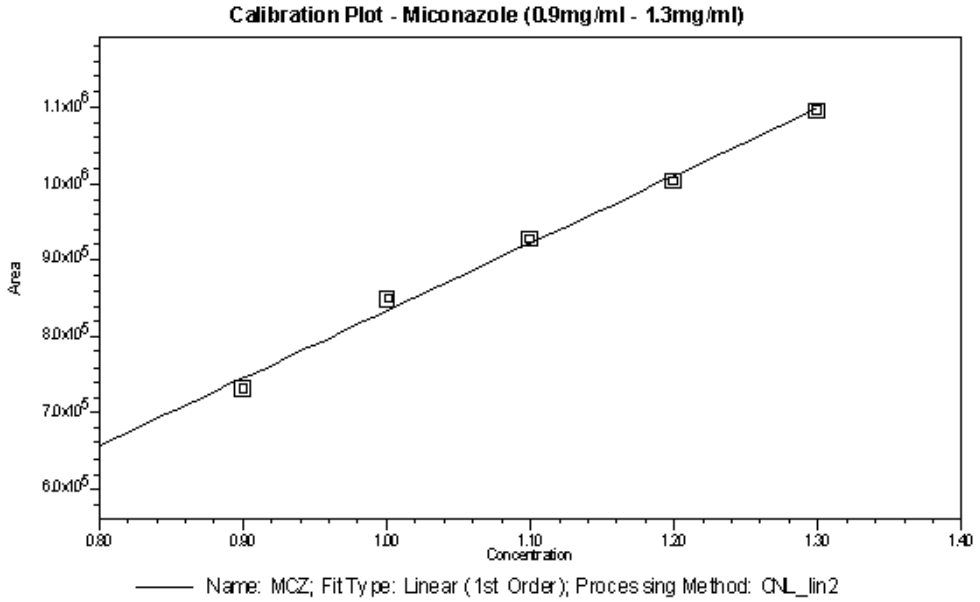


Figure 5b. Miconazole
Linearity plot - All points

Linearity Standard Report_CNL

Sample Set Name: CNL_MCZ_Lin_study2_062108	Acquired By: CLechnou
Project Name: Titusville NJ\Training\C. Lechnou Reseach	Date Printed: Monday, July 14, 200
Result Set Id: 4140	Calibration Id: 4141

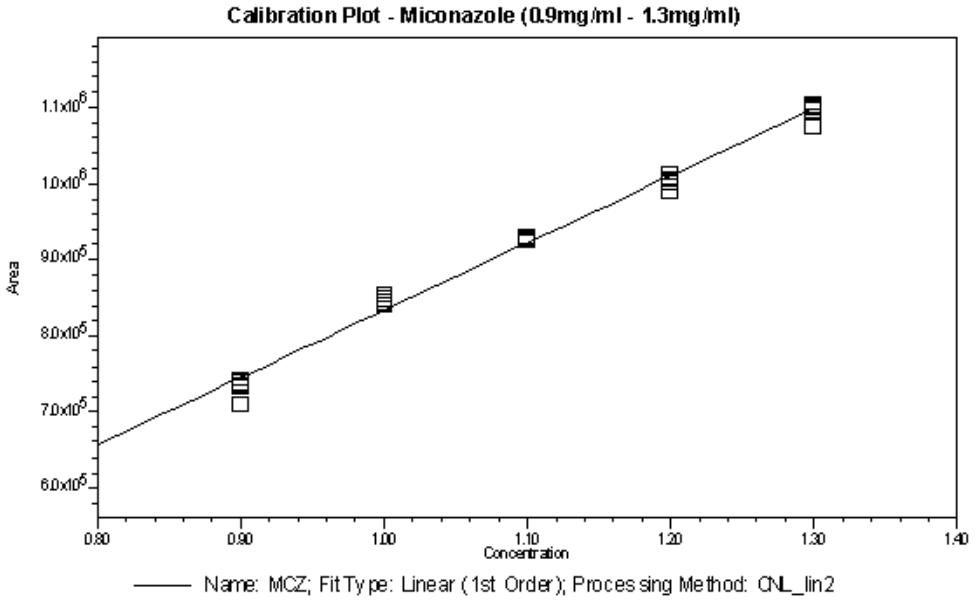


Table 13. (5-fluorocytosine – check standard analysis (5-fluorocytosine_std_check062508)

Sample Name	Injection number	Result ID	Retention Time	Area
5-fluorocytosine – 1.0mg/ml (check)	1	6302	2.28	1214582
5-fluorocytosine – 1.0mg/ml (check)	2	6303	2.28	1216093
5-fluorocytosine – 1.0mg/ml (check)	3	6304	2.28	1208542
5-fluorocytosine – 1.0mg/ml (check)	4	6305	2.28	1212621
5-fluorocytosine – 1.0mg/ml (check)	5	6306	2.27	1219786
Mean				1214307
%RSD				0.3

Sample Name	Injection number	Result ID	Retention Time	Area
5-fluorocytosine – 1.0mg/ml	1	6307	2.28	1189910
5-fluorocytosine – 1.0mg/ml	2	6308	2.28	1186187
Mean				1188263
%RSD				0.2

Note: for this analysis voltage and injection volume different than linearity analysis

(average areas used for calculations)
5-fluorocytosine standard weight = 500.61mg/250ml volumetric flask
5-fluorocytosine check standard weight = 100.44mg/50ml volumetric flask
Standard Agreement = 98.2%

Figure 6. (5-fluorocytosine 1.0mg/ml – check standard)

Sample Set Name: 5fluorocytosine_stdcheck062508 Acquired By: CLechnou
Project Name: Titusville NJ\Training\C. Lechnou Reseach Date Printed: Tuesday, July 15, 200
Result Set Id: 6299 Calibration Id: 6285

Acq Method Set: CE_MS System Name: TI_CE_Beckman
Date Acquired: Wednesday, June 25, 2008 3:37:27 PM EDT Channel Description:
Date Processed: Tuesday, July 15, 2008 10:09:02 AM EDT Channel Id: 4464
Processing Method: 5_Fluorocytosine_PM_LIN Result Id: 6302
SampleName: 5-fluorocytosine (check) Vial: 2 Injection: 1

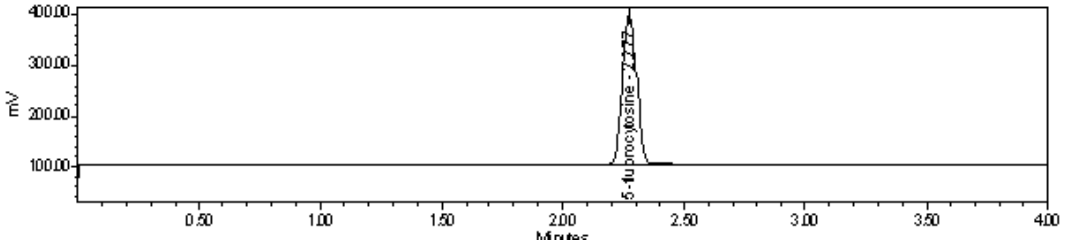


Table 14. (5-fluorocytosine Linearity Study - 5fluorocytosine_linstudy_062608)

Sample Name	Injection number	Result ID	Retention Time	Area
5-fluorocytosine – 0.7mg/ml	1	4648	1.87	725313
5-fluorocytosine – 0.7mg/ml	2	4649	1.86	721134
5-fluorocytosine – 0.7mg/ml	3	4650	1.85	712320
5-fluorocytosine – 0.7mg/ml	4	4651	1.85	709312
5-fluorocytosine – 0.7mg/ml	5	4652	1.85	707725
Mean				715161
% RSD				1.1

Table 15. (5-fluorocytosine Linearity Study)

Sample Name	Injection number	Result ID	Retention Time	Area
5-fluorocytosine – 0.8mg/ml	1	4653	1.84	780866
5-fluorocytosine – 0.8mg/ml	2	4654	1.84	780599
5-fluorocytosine – 0.8mg/ml	3	4655	1.84	790593
5-fluorocytosine – 0.8mg/ml	4	4656	1.84	789084
5-fluorocytosine – 0.8mg/ml	5	4657	1.84	787709
Mean				785770
% RSD				0.6

Table 16. (5-fluorocytosine Linearity Study)

Sample Name	Injection number	Result ID	Retention Time	Area
5-fluorocytosine – 0.9mg/ml	1	4658	1.85	878242
5-fluorocytosine – 0.9mg/ml	2	4659	1.84	874627
5-fluorocytosine – 0.9mg/ml	3	4660	1.84	873882
5-fluorocytosine – 0.9mg/ml	4	4661	1.85	891805
5-fluorocytosine – 0.9mg/ml	5	4662	1.85	884914
Mean				880694
%RSD				0.9

Table 17. (5-fluorocytosine Linearity Study)

Sample Name	Injection number	Result ID	Retention Time	Area
5-fluorocytosine – 1.0mg/ml	1	4663	1.84	951970
5-fluorocytosine – 1.0mg/ml	2	4664	1.84	952122
5-fluorocytosine – 1.0mg/ml	3	4665	1.84	961131
5-fluorocytosine – 1.0mg/ml	4	4666	1.84	957239
5-fluorocytosine – 1.0mg/ml	5	4667	1.84	957123
Mean				955917
% RSD				0.4

Table 18. (5-fluorocytosine Linearity Study)

Sample Name	Injection number	Result ID	Retention Time	Area
5-fluorocytosine – 1.1mg/ml	1	4668	1.86	1048998
5-fluorocytosine – 1.1mg/ml	2	4668	1.85	1049171
5-fluorocytosine – 1.1mg/ml	3	4669	1.85	1050947
5-fluorocytosine – 1.1mg/ml	4	4670	1.85	1049168
5-fluorocytosine – 1.1mg/ml	5	4671	1.85	1043723
Mean				1048401
% RSD				0.3

Table 19. (5-fluorocytosine Linearity Study)

Sample Name	Injection number	Result ID	Retention Time	Area
5-fluorocytosine – 1.2mg/ml	1	4672	1.85	1121913
5-fluorocytosine – 1.2mg/ml	2	4673	1.84	1129431
5-fluorocytosine – 1.2mg/ml	3	4674	1.84	1120289
5-fluorocytosine – 1.2mg/ml	4	4675	1.84	1112892
5-fluorocytosine – 1.2mg/ml	5	4676	1.84	1114526
Mean				1119810
%RSD				0.6

Table 20. (5-fluorocytosine Linearity Study)

Sample Name	Injection number	Result ID	Retention Time	Area
5-fluorocytosine – 1.3mg/ml	1	4677	1.84	1194536
5-fluorocytosine – 1.3mg/ml	2	4678	1.84	1183564
5-fluorocytosine – 1.3mg/ml	3	4679	1.83	1188725
5-fluorocytosine – 1.3mg/ml	4	4680	1.83	1186047
5-fluorocytosine – 1.3mg/ml	5	4681	1.83	1183030
Mean				1187198
%RSD				0.4

Figure 7a. 5-fluorocytosine
Linearity study

Linearity Standard Report_CNL2

Sample Set Name: 5fluorocytosine_linstudy 062608
Project Name: Titusville NJ\Training\C. Lechnou Reseach
Result Set Id: 4645

Acquired By: CLechnou
Date Printed: Tuesday, July 15, 200
Calibration Id: 4646

Component Results

	Equation	Name	RT	Area (µV*sec)	R^2	Concentration
1	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.874	725313	0.997917	0.701
2	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.857	721134	0.997917	0.701
3	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.854	712320	0.997917	0.701
4	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.853	709312	0.997917	0.701
5	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.853	707725	0.997917	0.701
6	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.841	780866	0.997917	0.801
7	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.840	780599	0.997917	0.801
8	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.840	790593	0.997917	0.801
9	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.842	789084	0.997917	0.801
10	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.842	787709	0.997917	0.801
11	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.851	878242	0.997917	0.900
12	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.845	874627	0.997917	0.900
13	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.843	873882	0.997917	0.900
14	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.847	891805	0.997917	0.900
15	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.847	884914	0.997917	0.900
16	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.844	951970	0.997917	1.001
17	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.842	952122	0.997917	1.001
18	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.840	961131	0.997917	1.001
19	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.839	957239	0.997917	1.001
20	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.839	957123	0.997917	1.001
21	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.858	1048998	0.997917	1.100
22	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.853	1049171	0.997917	1.100
23	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.850	1050947	0.997917	1.100

Figure 7b. 5-fluorocytosine
Linearity study

Linearity Standard Report_CNL2

Sample Set Name: 5fluorocytosine_linstudy 062608
Project Name: Titusville NJ\Training\C. Lechnou Reseach
Result Set Id: 4645

Acquired By: CLechnou
Date Printed: Tuesday, July 15, 200
Calibration Id: 4646

Component Results

	Equation	Name	RT	Area (µV*sec)	R^2	Concentration
24	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.848	1049168	0.997917	1.100
25	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.849	1043723	0.997917	1.100
26	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.846	1121913	0.997917	1.201
27	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.842	1129431	0.997917	1.201
28	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.840	1120289	0.997917	1.201
29	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.842	1112892	0.997917	1.201
30	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.840	1114526	0.997917	1.201
31	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.844	1194536	0.997917	1.300
32	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.840	1183654	0.997917	1.300
33	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.834	1188725	0.997917	1.300
34	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.835	1186047	0.997917	1.300
35	Y = 8.05e+005 X + 1.50e+005	5-fluorocytosine	1.834	1183030	0.997917	1.300

Component Results

	R	Residual SumofSquares	Equation
1	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
2	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
3	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
4	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
5	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
6	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
7	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
8	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
9	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
10	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005

Figure 7c. 5-fluorocytosine Linearity study

Linearity Standard Report_CNL2

Sample Set Name: 5fluorocytosine_linstudy 062608
Project Name: Titusville NJ\Training\C. Lechnou Reseach
Result Set Id: 4645

Acquired By: CLechnou
Date Printed: Tuesday, July 15, 200
Calibration Id: 4646

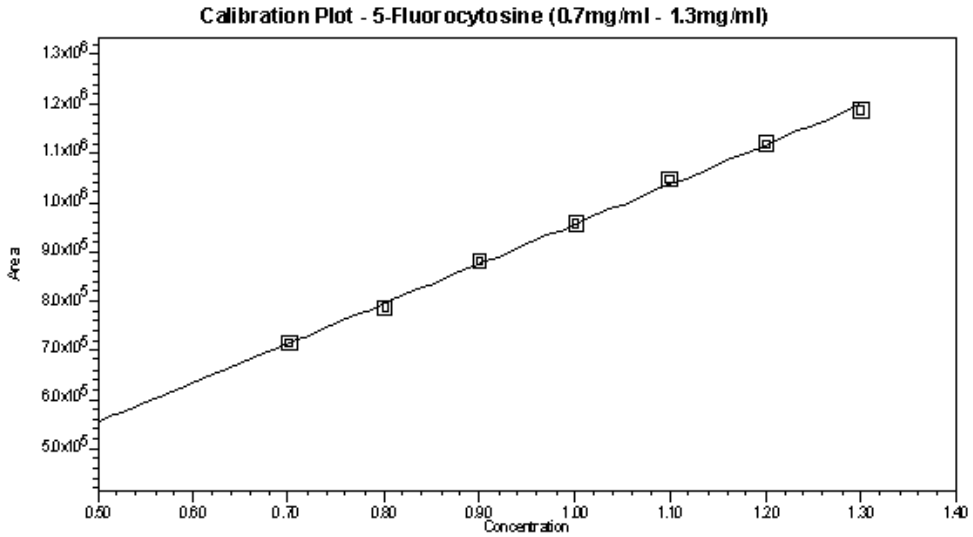
Component Results

	R	Residual SumofSquares	Equation
11	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
12	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
13	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
14	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
15	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
16	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
17	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
18	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
19	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
20	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
21	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
22	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
23	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
24	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
25	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
26	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
27	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
28	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
29	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
30	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
31	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
32	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
33	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
34	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005
35	0.998958	3.779793e+008	Y = 8.05e+005 X + 1.50e+005

Figure 8a. 5-fluorocytosine
Linearity plot - average points

Linearity Standard Report_CNL2

Sample Set Name: 5fluorocytosine_linstudy062608	Acquired By: CLechnou
Project Name: Titusville NJ\Training\C. Lechnou Reseach	Date Printed: Tuesday, July 15, 200
Result Set Id: 4645	Calibration Id: 4646



Name: 5-fluorocytosine; Fit Type: Linear (1st Order); Processing Method: 5_Fluorocytosine_PM_LIN

Figure 8b. 5-fluorocytosine
Linearity plot - all points

Linearity Standard Report_CNL2

Sample Set Name: 5fluorocytosine_linstudy 062608	Acquired By: CLechnou
Project Name: Titusville NJ\Training\C. Lechnou Reseach	Date Printed: Tuesday, July 15, 200
Result Set Id: 4645	Calibration Id: 4646

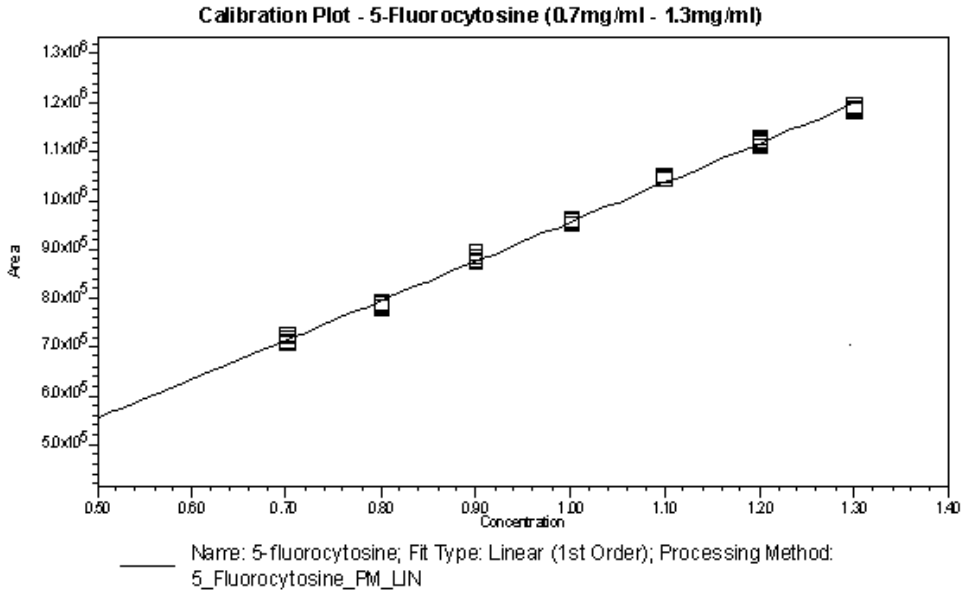
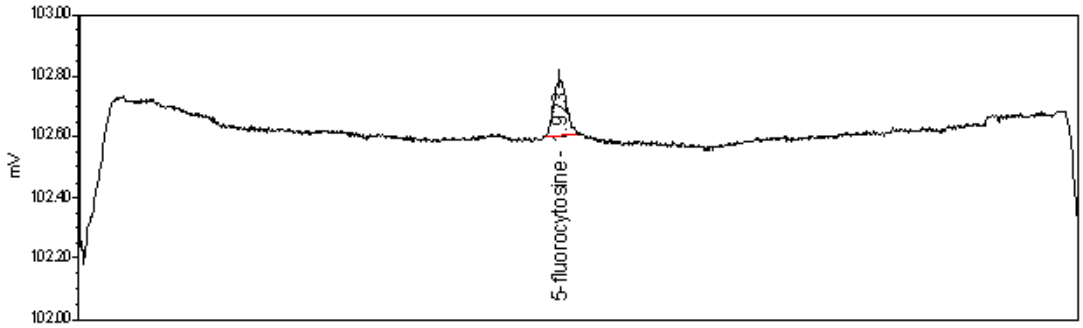


Figure 9. 5-fluorocytosine LOD chromatogram

Signal to Noise Report_5FC

Sample Set Name: 5fluorocytosine_LOD_071308
Acquired By: CLechnou
Project Name: Titusville NJ\Training\C. Lechnou Reseach
Date Printed: Tuesday, July 15, 20(
Result Set Id: 6321
Calibration Id: 6322

Acq Method Set: CE_MS
System Name: TI_CE_Beckman
Date Acquired: Sunday, July 13, 2008 11:18:36 AM EDT
Channel Description:
Date Processed: Tuesday, July 15, 2008 10:44:08 AM EDT
Channel Id: 6216
Processing Method: 5_Fluorocytosine_PM_LOD1
Result Id: 6326
SampleName: 5-fluorocytosine_0.0005
Vial: 2
Injection: 1



$$S/N = \frac{11}{3} = 4 \text{ mm}$$

Table 21. (5-fluorocytosine LOD/LOQ study)

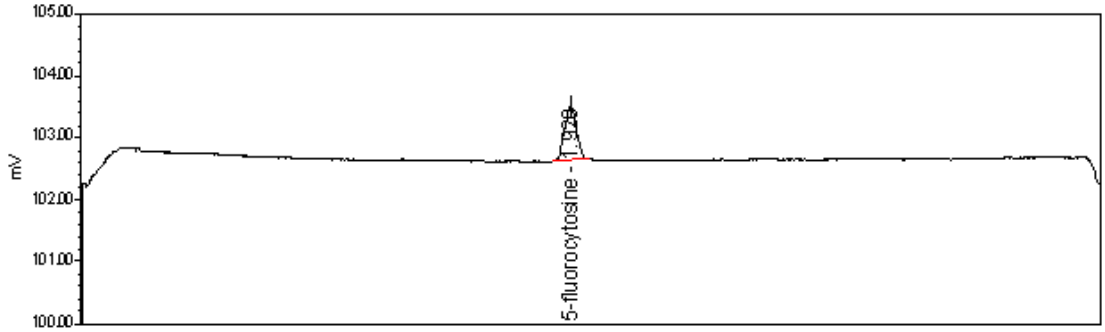
Sample Name	Injection number	Result ID	Retention Time	Area
5-fluorocytosine – 0.0005mg/ml	1	6326	1.92	683
5-fluorocytosine – 0.0005mg/ml	2	6327	1.94	974
5-fluorocytosine – 0.0005mg/ml	3	6328	1.92	1170
Mean				943
%RSD				26.0

Figure 10. 5-fluorocytosine LOQ chromatogram

Signal to Noise Report

Sample Set Name: 5fluorocytosine_LOQ_071308
Acquired By: CLechnou
Project Name: Titusville NJ\Training\C. Lechnou Reseach
Date Printed: Tuesday, July 15, 200
Result Set Id: 6291
Calibration Id: 6292

Acq Method Set: CE_MS
System Name: TI_CE_Beckman
Date Acquired: Sunday, July 13, 2008 11:46:11 AM EDT
Channel Description:
Date Processed: Sunday, July 13, 2008 3:56:07 PM EDT
Channel Id: 6225
Processing Method: 5_Fluorocytosine_PM_LOD
Result Id: 6294
SampleName: 5-fluorocytosine_0.0025
Vial: 1
Injection: 1



$$S/N = \frac{11}{1} = 11 \text{ mm}$$

Table 22. (5-fluorocytosine LOD/LOQ study)

Sample Name	Injection number	Result ID	Retention Time	Area
5-fluorocytosine – 0.0025mg/ml	1	6294	1.92	3047
5-fluorocytosine – 0.0025mg/ml	2	6295	1.92	2859
5-fluorocytosine – 0.0025mg/ml	3	6296	1.92	2898
Mean				2935
%RSD				3.4

Table 23. (Nystatin – check standard analysis- nystatin_check_std_070308)

Sample Name	Injection number	Result ID	Retention Time	Area
Nystatin 0.5mg/ml	1	6344	1.55	2618910
Nystatin 0.5mg/ml	2	6345	1.54	2579197
Mean				2599053
%RSD				1.1

Sample Name	Injection number	Result ID	Retention Time	Area
Nystatin 0.5mg/ml (check)	1	6347	1.53	2690856
Nystatin 0.5mg/ml (check)	2	6348	1.53	2651320
Nystatin 0.5mg/ml (check)	3	6349	1.53	2622331
Nystatin 0.5mg/ml (check)	4	6350	1.53	2660241
Nystatin 0.5mg/ml (check)	5	6351	1.53	2632332
Mean				2651416
%RSD				1.0

Nystatin standard weight = 500.89 mg/250ml volumetric flask

Nystatin check standard weight = 100.18mg/50ml volumetric flask

Standard Agreement = 98.0%

Table 24. (Nystatin Linearity Study)

Sample Name	Injection number	Result ID	Retention Time	Area
Nystatin – 0.00025mg/ml	1	5181	1.64	13087
Nystatin – 0.00025mg/ml	2	5182	1.65	13160
Nystatin – 0.00025mg/ml	3	5183	1.64	13025
Nystatin – 0.00025mg/ml	4	5184	1.64	13080
Nystatin – 0.00025mg/ml	5	5185	1.64	13003
Mean				13071
% RSD				0.5

Table 25. (Nystatin Linearity Study)

Sample Name	Injection number	Result ID	Retention Time	Area
Nystatin – 0.1mg/ml	1	5186	1.62	940542
Nystatin – 0.1mg/ml	2	5187	1.63	943536
Nystatin – 0.1mg/ml	3	5188	1.62	931127
Nystatin – 0.1mg/ml	4	5189	1.62	942290
Nystatin – 0.1mg/ml	5	5190	1.61	932920
Mean				938083
% RSD				0.6

Table 26. (Nystatin Linearity Study)

Sample Name	Injection number	Result ID	Retention Time	Area
Nystatin – 0.3mg/ml	1	5191	1.64	1564255
Nystatin – 0.3mg/ml	2	5192	1.65	1573456
Nystatin – 0.3mg/ml	3	5193	1.65	1590276
Nystatin – 0.3mg/ml	4	5194	1.65	1606906
Nystatin – 0.3mg/ml	5	5195	1.65	1607174
Mean				1588414
%RSD				1.2

Table 27. (Nystatin Linearity Study)

Sample Name	Injection number	Result ID	Retention Time	Area
Nystatin – 0.5mg/ml	1	5196	1.73	2067477
Nystatin – 0.5mg/ml	2	5197	1.73	2128022
Nystatin – 0.5mg/ml	3	5198	1.74	2091569
Nystatin – 0.5mg/ml	4	5199	1.74	2097015
Nystatin – 0.5mg/ml	5	5200	1.75	2100001
Mean				2096817
% RSD				1.0

Table 28. (Nystatin Linearity Study)

Sample Name	Injection number	Result ID	Retention Time	Area
Nystatin – 0.7mg/ml	1	5201	1.74	2429941
Nystatin – 0.7mg/ml	2	5202	1.74	2342039
Nystatin – 0.7mg/ml	3	5203	1.74	2358212
Nystatin – 0.7mg/ml	4	5204	1.73	2357520
Nystatin – 0.7mg/ml	5	5205	1.73	2409336
Mean				2379409
% RSD				1.6

Table 29. (Nystatin Linearity Study)

Sample Name	Injection number	Result ID	Retention Time	Area
Nystatin – 0.9mg/ml	1	5206	1.73	2555334
Nystatin – 0.9mg/ml	2	5207	1.73	2556081
Nystatin – 0.9mg/ml	3	5208	1.72	2460006
Nystatin – 0.9mg/ml	4	5209	1.73	2490099
Nystatin – 0.9mg/ml	5	5210	1.73	2463426
Mean				2504989
%RSD				1.9

Table 30. (Nystatin Linearity Study)

Sample Name	Injection number	Result ID	Retention Time	Area
Nystatin – 1.1mg/ml	1	5211	1.77	2993223
Nystatin – 1.1mg/ml	2	5212	1.77	2869904
Nystatin – 1.1mg/ml	3	5213	1.77	2904944
Nystatin – 1.1mg/ml	4	5214	1.77	2938168
Nystatin – 1.1mg/ml	5	5215	1.76	2840529
Mean				2909354
%RSD				

Figure 11. Nystatin
0.5mg/ml chromatogram

Chromatogram Report

Sample Set Name: Nystatin_lin_study_070408	Acquired By: CLechnou
Project Name: Titusville NJ\Training\C. Lechnou Reseach	Date Printed: Tuesday, July 15, 200
Result Set Id: 5178	Calibration Id: 5179

Acq Method Set: CE_MS	System Name: TI_CE_Beckman
Date Acquired: Friday, July 04, 2008 2:40:28 PM EDT	Channel Description:
Date Processed: Friday, July 04, 2008 9:59:55 PM EDT	Channel Id: 4850
Processing Method: Nystatin_PM_LIN	Result Id: 5196
SampleName: nystatin-0.5mg/ml	Vial: 6 Injection: 1

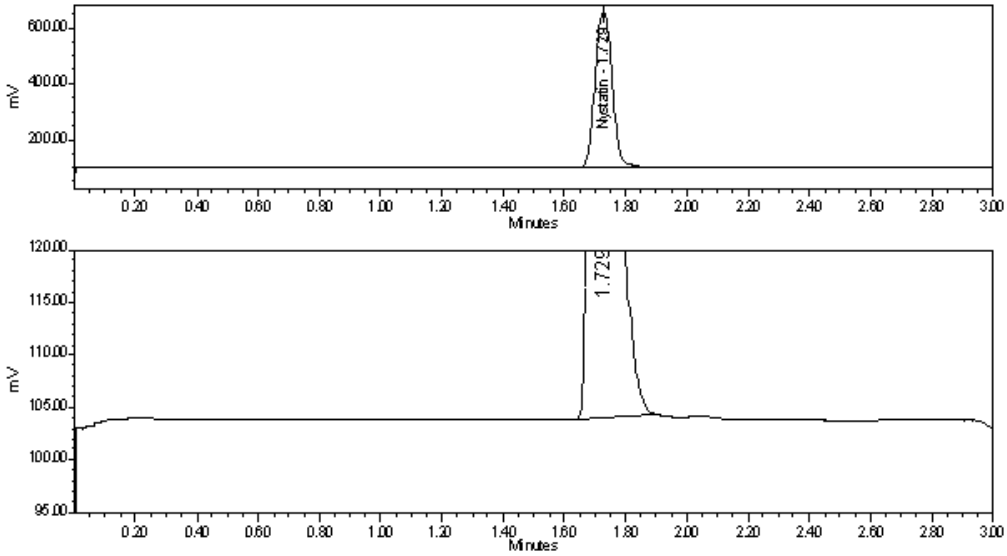


Figure 12a. Nystatin Linearity Study

Linearity Standard Report_CNL2

Sample Set Name: Nystatin_lin_study_070408
Project Name: Titusville NJ\Training\C. Lechnou Reseach
Result Set Id: 5178

Acquired By: CLechnou
Date Printed: Sunday, July 20, 200
Calibration Id: 5179

Component Results

	Equation	Name	RT	Area (µV*sec)	R^2	Concentration
1	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.638	13087	0.891021	0.00025
2	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.646	13160	0.891021	0.00025
3	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.640	13025	0.891021	0.00025
4	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.637	13080	0.891021	0.00025
5	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.638	13003	0.891021	0.00025
6	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.624	940542	0.891021	0.10018
7	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.626	943536	0.891021	0.10018
8	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.622	931127	0.891021	0.10018
9	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.623	942290	0.891021	0.10018
10	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.614	932920	0.891021	0.10018
11	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.640	1564255	0.891021	0.30047
12	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.647	1573456	0.891021	0.30047
13	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.650	1590276	0.891021	0.30047
14	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.654	1606906	0.891021	0.30047
15	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.654	1607174	0.891021	0.30047
16	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.729	2067477	0.891021	0.50089
17	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.734	2128022	0.891021	0.50089
18	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.737	2091569	0.891021	0.50089
19	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.742	2097015	0.891021	0.50089
20	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.747	2100001	0.891021	0.50089
21	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.737	2429941	0.891021	0.70153
22	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.738	2342039	0.891021	0.70153
23	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.736	2358212	0.891021	0.70153

Figure 12b. Nystatin Linearity Study

Linearity Standard Report_CNL2

Sample Set Name: Nystatin_lin_study_070408
Project Name: Titusville NJ\Training\C. Lechnou Reseach
Result Set Id: 5178

Acquired By: CLechnou
Date Printed: Sunday, July 20, 200
Calibration Id: 5179

Component Results

	Equation	Name	RT	Area (µV*sec)	R^2	Concentration
24	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.731	2357520	0.891021	0.70153
25	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.732	2409336	0.891021	0.70153
26	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.730	2555334	0.891021	0.90088
27	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.727	2556081	0.891021	0.90088
28	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.724	2460006	0.891021	0.90088
29	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.726	2490099	0.891021	0.90088
30	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.726	2463426	0.891021	0.90088
31	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.766	2993223	0.891021	1.10086
32	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.766	2869904	0.891021	1.10086
33	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.766	2904944	0.891021	1.10086
34	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.767	2938168	0.891021	1.10086
35	Y = 2.32e+006 X + 5.78e+005	Nystatin	1.765	2840529	0.891021	1.10086

Component Results

	R	Residual SumofSquares	Equation
1	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
2	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
3	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
4	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
5	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
6	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
7	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
8	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
9	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
10	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005

Figure 12c. Nystatin Linearity Study

Linearity Standard Report_CNL2

Sample Set Name: Nystatin_lin_study_070408
Project Name: Titusville NJ\Training\C. Lechnou Reseach
Result Set Id: 5178

Acquired By: CLechnou
Date Printed: Sunday, July 20, 200
Calibration Id: 5179

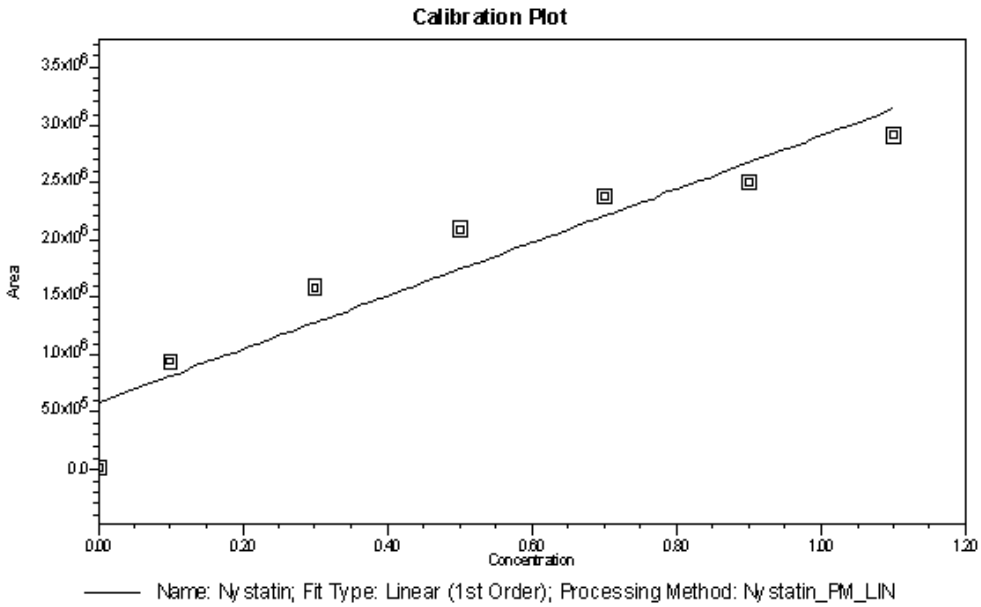
Component Results

	R	Residual Sum of Squares	Equation
11	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
12	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
13	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
14	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
15	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
16	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
17	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
18	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
19	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
20	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
21	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
22	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
23	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
24	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
25	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
26	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
27	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
28	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
29	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
30	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
31	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
32	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
33	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
34	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005
35	0.943939	6.678384e+011	Y = 2.32e+006 X + 5.78e+005

Figure 13. Nystatin Linearity Plot -average points

Linearity Standard Report_CNL2

Sample Set Name: Nystatin_lin_study_070408	Acquired By: CLechnou
Project Name: Titusville NJ\Training\C. Lechnou Reseach	Date Printed: Sunday, July 20, 200
Result Set Id: 5178	Calibration Id: 5179



CONCLUSION

The results of this study further support the validity of capillary electrophoresis as a potential alternative to HPLC when applicable. One of the greatest challenges for this analytical tool is injection precision. In comparison to HPLC, for method development an internal standard is commonly employed. In this study, we show that with careful attention to pre-injection capillary equilibration, column regeneration, and ratio of organic to solvent, respectable precision results comparable to HPLC are obtained. The sometimes-necessary use of organic as a solvent can potentially have adverse effects of reproducibility. The effect of the organic on the electroosmotic flow can be unpredictable and only be understood after experimentation with a particular organic and buffer system. In this study, both miconazole and nystatin were dissolved in methanol and dimethlyformamide respectively. As previously discussed, initially miconazole was prepared into a 50% organic solution for analysis. The %RSD values obtained from this condition were around 7%. For the final studies, miconazole was prepared with 25% organic, which produced %RSD results of less than 2% for all seven concentrations.

The findings were different for nystatin, due to this compounds lack of solubility in water. The low linearity R^2 results of 0.88 illustrated solubility problems with the final solutions at 75% aqueous solvent level.

Compounds that pose similar solubility challenges as Nystatin can provide more of a challenge for method development.

Since 5-fluorocytosine is soluble in water, capillary electrophoresis analysis is a good choice of an analytical tool. For this method, the run buffer and compound solvent were the same. Linearity and precision, LOQ, and LOD results were all very comparable to that of HPLC.

Miconazole and 5-fluorocytosine have shown that this technology can prove to be an alternative to HPLC. Although, in fairness to HPLC, an HPLC analysis can be very rugged with continuous use, with relative less care to variables that may cause issues for capillary electrophoresis during routine operation.

In conclusion, in the pursuit of adhering to stringent validation criteria that are utilized for HPLC, careful attention needs to be considered with solvent ratios, loss of buffer effectiveness, pre-injection capillary conditioning, and capillary regeneration. In addition, during routine analysis attention is needed to ensure source and

destination run buffer levels are comparable. If the necessary care is taken during method development and instrument set-up during analysis, for some compounds capillary electrophoresis provides another choice of analytical tool that offers many advantages over HPLC.

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