Haskell Laboratory for Toxicology
and Industrial Medicine

June 11, 2001

TO: MR [redacted] Pilot Developmental Toxicity Study in Rats

cc: L. A. Malley

FROM: J. C. Maslanka
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**ANALYSIS FOR [redacted] IN DOSING SOLUTIONS**

| | |
|---|---|
| Medical Research Project Number: | [redacted] |
| Haskell Sample Number: | 24768 |
| Analytical Reference: | 24768 |
| Analytical Report Number: | HA-2001-022 |
| Service Code: | |
| Notebook References: | [redacted] |

The analytical report for the study identified above is a summary of the mixing and stability for the [redacted] and will be used as reference for all studies under this MR.

[redacted] Pilot Developmental Toxicity Study in Rats

## ANALYSIS FOR [redacted] IN DOSING SOLUTIONS

Medical Research Project Number: [redacted] 24768
Haskell Sample Number:
Analytical Report Number: HA-2001-022

## SUMMARY

Dosing solutions at concentrations of 5.0, 10.0, 40.0 and 70.0 mg/mL of [redacted] were prepared and collected for uniformity/concentration verification and stability analysis (5-hour room temperature) on February 26, 2001. Dosing solutions from the same preparation of [redacted] were collected on the fourth day of refrigeration and analyzed for stability on March 1, 2001. Dosing solutions at the same concentrations were prepared and collected on March 7, 2001 for uniformity/concentration verification. On March 10, 2001, dosing solutions from the same preparation of [redacted] were collected on the fourth day of refrigeration to be analyzed for refrigeration stability/concentration along with 5-hour room temperature stability. In addition, 0 mg/mL (control) samples were submitted for analysis with the set of samples.

[redacted] as used in this report refers to the active ingredient (a.i.) in [redacted]
Concentrations of [redacted] in separate dosing solutions were measured by gas chromatography (GC).

The solution vehicle for the study was de-ionized water.

Results from analysis of the dosing solutions prepared and collected for uniformity/concentration verification and stability on February 26, 2001, indicated that test substance was mixed properly, at the expected concentration (± 17%) in all levels and stable in the vehicle for 5 hours at room temperature. Results from analysis of the dosing solutions collected on the fourth day of refrigeration from the same preparation indicated that the test substance was stable under these conditions. Results for the dosing solutions prepared and collected on March 7, 2001 for uniformity/concentration verification indicated that test substance was mixed properly and at the expected concentration (± 11%) at all levels. Results from analysis of the dosing solutions collected on the fourth day of refrigeration and then held 5 hours at room temperature from the same preparation indicated that the test substance was stable under these conditions.

[redacted] was not detected in the 0 mg/mL (control) sample.

## SAMPLE SUBMITTAL

All dosing solution samples were collected on the same day the solutions were prepared or as indicated by the stability analysis for the study. The solution vehicle was de-ionized water.

## METHODS

1. Analytical Methods

a. Dosing Solution Treatment

Samples containing [redacted] at the concentrations of 0, 5.0, 10.0, 40.0 and 70.0 mg/mL were collected on February 26, 2001 for uniformity/concentration verification and 5 hour stability. Samples from the same preparation were collected on March 1, 2001 for 4-day refrigerated stability/concentration analysis. Samples at the same concentrations were collected on March 7, 2001 for uniformity/concentration verification. Samples from the same preparation were collected on March 10, 2001 for 4-day refrigerated stability/concentration and 5 hour room temperature stability analysis.

Each dosing sample was analyzed after dilution with methanol and/or an equivalent amount of diluted control to an expected concentration of 2.5, 4.0, or 7.0 mg/mL (a.i.) prior to analysis.

Samples submitted for analysis were analyzed the day the solutions were received or frozen until analyzed by the testing group.

b. Chromatographic Conditions

| | |
|---|---|
| Instrument: | Hewlett-Packard Model 6890 GC |
| Column: | HP-1, 30 m x 0.32 mm ID, 0.25 $\mu$m film thickness |
| Injector: | Split, 250°C |
| Detector: | FID; 300°C |
| Carrier Gas: | Helium (2.7 mL/min) |
| Split vent: | 53.2 mL/min |
| Injection Volume: | 1 microliter |
| Oven Program: | Gradient |
| Initial Temperature: | 50°C |
| Initial Time | 0.0 min. |
| Level 1 Rate: | 40°C/min. |
| Level 1 Temperature: | 300°C |
| Run Time: | 22.25 minutes |

c. Calibration and Quantitation

A stock solution of the test material (a separate sample of [redacted]H-24768 used as analytical reference) was made in methanol. Before analysis of the samples collected for February 26, 2001 preparation, appropriate aliquots of the stock were diluted with the methanol to make calibration standards, which bracketed the target concentration of the diluted dosing samples. Before analysis of the remaining samples in the study, appropriate aliquots of the stock were diluted with the methanol after an equivalent amount of the diluted control to match the samples was added. This was used to make calibration standards, which bracketed the target concentration of the diluted dosing samples. Peak heights (4 retention times) from GC analysis of these standards were used to construct a calibration curve by least square regression (see Figure 1a - 1d for a representative calibration curves). Measured concentrations for dosing solutions were determined by applying the peak heights from replicate injections of the samples to each calibration curve. The mean result from the concentrations at all retention times (n = 4) is reported as the concentration for the sample.

Test substance uniformity in the vehicle was evaluated by calculating the coefficient of variation (C.V. = standard deviation/mean x 100) of the measured concentrations in duplicate samples for each dosing level. A coefficient of variation of less than 10% is the standard criterion at Haskell Laboratory for acceptable distribution of the test substance throughout the solution. The mean result of the duplicate samples for each dosing level was used to determine the concentration of the test substance for the respective dosing levels.

Stability was evaluated by using the mean of the duplicate samples for concentration verification as the baseline for comparing the corresponding room temperature and refrigerated results.

## ANALYTICAL RESULTS

### A. Chromatography

[redacted] eluted from the GC column as resolved peaks over retention time range of approximately 5 to 20 minutes. For the purpose of quantitation, the retention times of 7.9, 8.2, 8.5, and 9.0 minutes were determined to be representative of the [redacted] in the dosing matrix. Representative GC chromatograms are shown in Figures 2(a - c). Test substance was not detected in the 0 mg/mL control.

### B. Concentration Verification and Stability Samples

Analytical results from dosing solutions prepared February 26, 2001, and March 7, 2001 and analyzed for concentration verification and stability are shown in Table I and Summary Table 1.

The following table summarizes the results for uniformity/concentration verification and stability analyses for the February 26, 2001 preparation.

| Preparation Date | Nominal mg/mL | Measured[a] mg/mL | Average % Nominal | CV % | Stability[b] % Nominal | Stability[c] % Nominal |
|---|---|---|---|---|---|---|
| 26-Feb-01 | 5.0 | 4.37, 4.23 | 86.0 | 2 | 83.7 | 100.2 |
| | 10.0 | 8.40, 8.39 | 83.9 | 0.1 | 80.5 | 93.1 |
| | 40.0 | 34.6, 32.0 | 83.3 | 5 | 79.0 | 94.5 |
| | 70.0 | 61.0, 61.7 | 87.7 | 1 | 84.5 | 93.5 |

a Duplicate samples analyzed.
b Stability samples held for 5 hours at room temperature.
c Stability samples held for 4 days refrigerated.

The results for samples prepared on February 26, 2001, show that the test substance was adequately mixed (CV's less than 10), at the targeted levels (target = ± 17% of nominal) and stable in the vehicle when held 5 hours at room temperature and for 4 days refrigerated. All reported values for these samples are lower than expected and was due to the analytical standards being prepared in methanol without the matrix correction. The method was modified to include matrix correction for the 4-day refrigerated samples and all results are within ± 10% of nominal. Test substance was not detected in the 0 mg/mL sample.

The following table summarizes the results for concentration verification and stability analyses for the March 7, 2001 preparation.

| Preparation Date | Nominal mg/mL | Measured[a] mg/mL | Average % Nominal | CV % | Stability[b] % Nominal | Stability[c] % Nominal |
|---|---|---|---|---|---|---|
| 7-Mar-01 | 5.0 | 4.72, 4.54 | 92.6 | 3 | 101.2 | 107.4 |
| | 10.0 | 9.36, 8.75 | 90.5 | 5 | 97.0 | 97.2 |
| | 40.0 | 40.2, 38.9 | 98.9 | 2 | 105.2 | 103.3 |
| | 70.0 | 63.2, 61.7 | 89.1 | 2 | 120.3 | 99.6 |

a Duplicate samples analyzed.
b Stability samples held for 4 days refrigerated.
c Stability samples held for 4 days refrigerated, then 5 hours at room temperature.

The results for samples prepared on March 7, 2001, show that the test substance was adequately mixed (CV's less than 10), at the targeted levels (target = ± 11% of nominal) and stable in the vehicle when held 4 days refrigerated and followed by 5 hours at room temperature. The result for the 70.0 mg/mL, 4-day refrigerated sample is higher than expected (120% of nominal) but is due to sampling and analytical variability. All other levels in this sampling were acceptable for the 4-day refrigerated stability. Test substance was not detected in the 0 mg/mL sample.

## C. Conclusion

Results from the analysis of the samples during the study indicate that the test substance was mixed properly, at the targeted levels and stable under the conditions of the study. Test substance was not found in the 0 mg/mL samples.

## ACKNOWLEGEMENTS

Samples analysis by Sheila A. Riley, (Chemistry Associate) and Janet C. Maslanka (Chemist).

## SIGNATURES

Report by: Janet C. Maslanka — 11-June-2001
Janet C. Maslanka, Chemist; Day/Mo/Yr

Date issued: 11-June-2001

Table I. Concentration Verification and Stability of [redacted] Dosing Solutions

| Preparation Date<br>Sample Type | mg/mL [redacted]<br>Nominal | <br>Measured | Percent<br>Nominal |
|---|---|---|---|
| 26-Feb-2001 | | | |
| Concentration Verification(A) | | | |
| Control | 0.0 | ND(B) | ---- |
| | 5.0-1 | 4.37 | 87.4 |
| | 5.0-2(C) | 4.23 | 84.6 |
| | *Mean(D):* | *4.30±0.1* | *(86.0)* |
| | | *C.V. 2%* | |
| | 10.0-1(C) | 8.40 | 84.0 |
| | 10.0-2 | 8.39 | 83.9 |
| | *Mean(D):* | *8.39±0.01* | *(83.9)* |
| | | *C.V. 0.1%* | |
| | 40.0-1(C) | 34.6 | 86.5 |
| | 40.0-2 | 32.0 | 80.0 |
| | *Mean(D):* | *33.3±1.8* | *(83.3)* |
| | | *C.V. 5%* | |
| | 70.0-1 | 61.0 | 87.1 |
| | 70.0-2(C) | 61.7 | 88.1 |
| | *Mean(D):* | *61.4±0.5* | *(87.7)* |
| | | *C.V. 1%* | |
| Stability | | | |
| 5 Hour Room Temperature | 5.0 | 4.18 | 83.7 |
| 4-Day Refrigerated(E) | 5.0 | 5.01 | 100.2 |
| 5 Hour Room Temperature | 10.0 | 8.05 | 80.5 |
| 4-Day Refrigerated(E) | 10.0 | 9.31 | 93.1 |
| 5 Hour Room Temperature | 40.0 | 31.6 | 79.0 |
| 4-Day Refrigerated(E) | 40.0 | 37.8 | 94.5 |
| 5 Hour Room Temperature | 70.0 | 59.2 | 84.5 |
| 4-Day Refrigerated(E) | 70.0 | 65.5 | 93.5 |

(A) Duplicate samples per level were analyzed. Mean, S.D. and C.V. calculated to verify uniformity of mixture.
(B) Denotes not detected.
(C) Mean result of the original analysis and the original sample reanalyzed.
(D) Mean result of the all analyses for the duplicate samples.
(E) Mean result of duplicate submitted samples reported.

Table I. (continued) Concentration Verification and Stability of [redacted] in Dosing Solutions

| Preparation Date / Sample Type | mg/ml [redacted] Nominal | | Measured | Percent Nominal |
|---|---|---|---|---|
| 7-Mar-2001 | | | | |
| Concentration Verification(A) | | | | |
| Control | 0.0 | | ND(B) | ---- |
| | 5.0-1 | | 4.72 | 94.4 |
| | 5.0-2 | | 4.54 | 90.8 |
| | | *Mean:* | *4.63±0.1* | *(92.6)* |
| | | | *C.V. 3%* | |
| | 10.0-1 | | 9.36 | 93.6 |
| | 10.0-2 | | 8.75 | 87.5 |
| | | *Mean:* | *9.05±0.4* | *(90.5)* |
| | | | *C.V. 5%* | |
| | 40.0-1 | | 40.2 | 100.5 |
| | 40.0-2 | | 38.9 | 97.3 |
| | | *Mean:* | *39.6±0.9* | *(98.9)* |
| | | | *C.V. 2%* | |
| | 70.0-1 | | 63.2 | 90.2 |
| | 70.0-2 | | 61.7 | 88.1 |
| | | *Mean:* | *62.4±1.1* | *(89.1)* |
| | | | *C.V. 2%* | |
| Stability | | | | |
| 4-Day Refrigerated | 5.0 | | 5.06 | 101.2 |
| 5 Hour Room Temperature(C) | 5.0 | | 5.37 | 107.4 |
| 4-Day Refrigerated | 10.0 | | 9.70 | 97.0 |
| 5 Hour Room Temperature(C) | 10.0 | | 9.72 | 97.2 |
| 4-Day Refrigerated | 40.0 | | 42.1 | 105.2 |
| 5 Hour Room Temperature(C) | 40.0 | | 41.3 | 103.3 |
| 4-Day Refrigerated | 70.0 | | 84.2 | 120.3 |
| 5 Hour Room Temperature(C) | 70.0 | | 69.7 | 99.6 |

(A) Duplicate samples per level were analyzed. Mean, S.D. and C.V. calculated to verify uniformity of mixture.
(B) Denotes not detected.
(C) Samples held for 4 days refrigerated, then 5 hours at room temperature.

**Figure 1**
**Representative Analytical Calibration Curve**

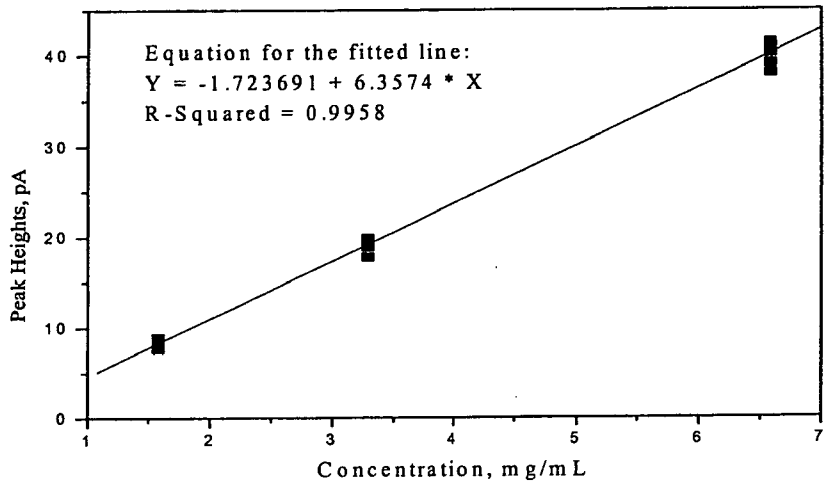


**Figure 1a**: Calibration curve (R.T. = 7.9 minutes) showing linear fit (line) to replicate peak height measurements (squares) for calibration solutions of [redacted] diluted over a concentration range of 1.579 to 6.58 mg/mL.

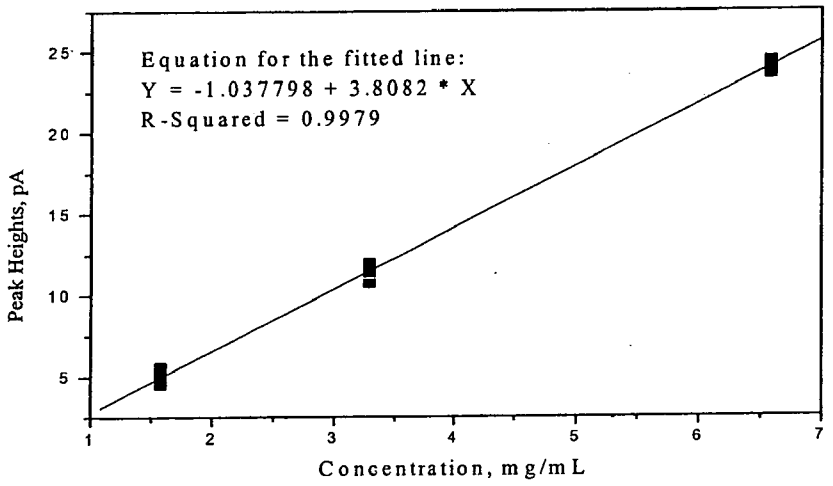


**Figure 1b**: Calibration curve (R.T. = 8.2 minutes) showing linear fit (line) to replicate peak height measurements (squares) for calibration solutions of [redacted] diluted over a concentration range of 1.579 to 6.58 mg/mL.

**Figure 1 (continued)**
**Representative Analytical Calibration Curve**

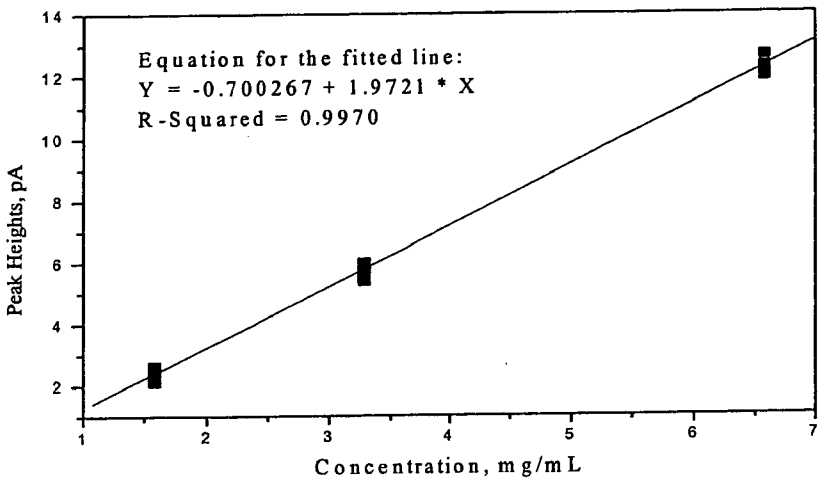


**Figure 1c**: Calibration curve (R.T. = 8.5 minutes) showing linear fit (line) to replicate peak height measurements (squares) for calibration solutions of [redacted] diluted over a concentration range of 1.579 to 6.58 mg/mL.

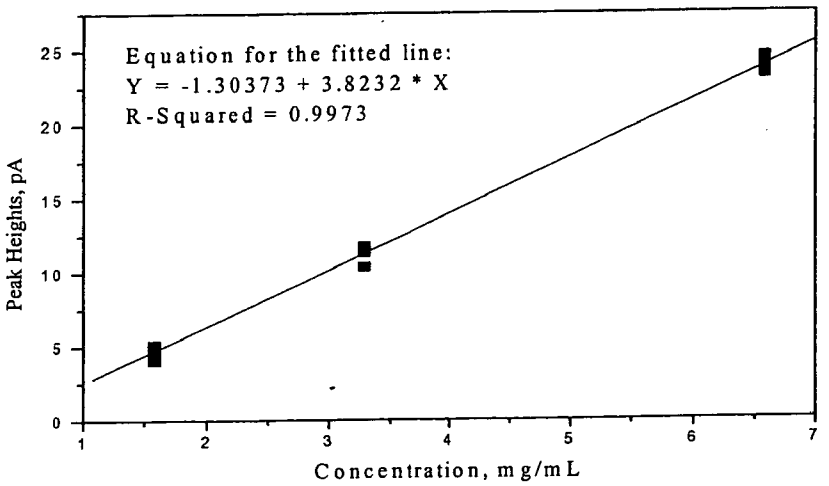


**Figure 1d**: Calibration curve (R.T. = 9.0 minutes) showing linear fit (line) to replicate peak height measurements (squares) for calibration solutions of [redacted] diluted over a concentration range of 1.579 to 6.58 mg/mL.

## Figure 2
## Representative Gas Chromatography Chromatograms

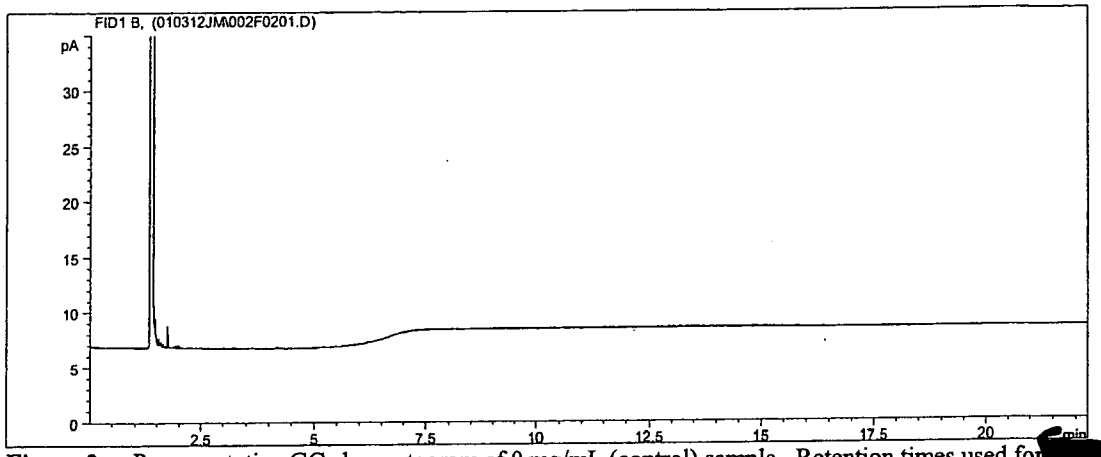


**Figure 2a:** Representative GC chromatogram of 0 mg/mL (control) sample. Retention times used for [redacted] are approximately 7.9, 8.2, 8.5, and 9.0 minutes.

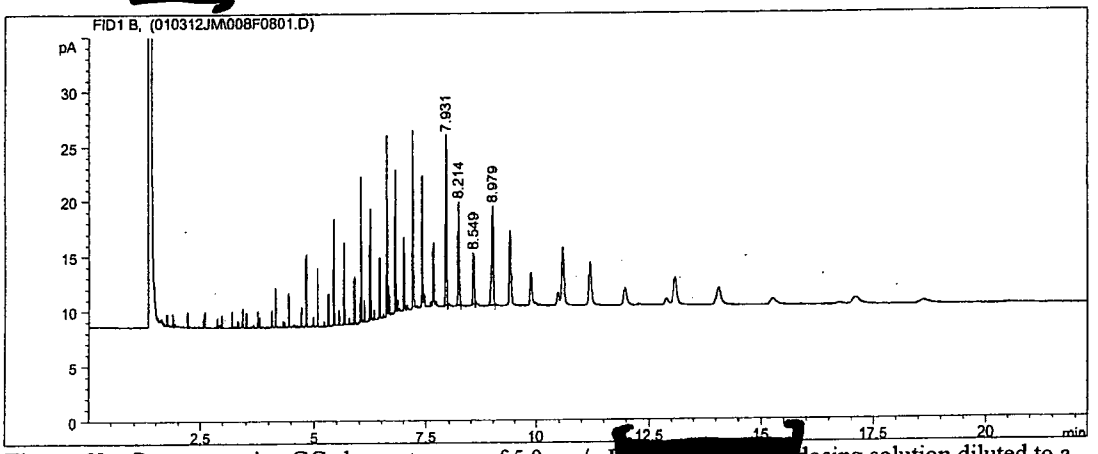


**Figure 2b:** Representative GC chromatogram of 5.0 mg/mL [redacted] dosing solution diluted to a nominal concentration of 2.5 mg/mL. The measured concentration of the representative solution is 5.36 mg/mL.

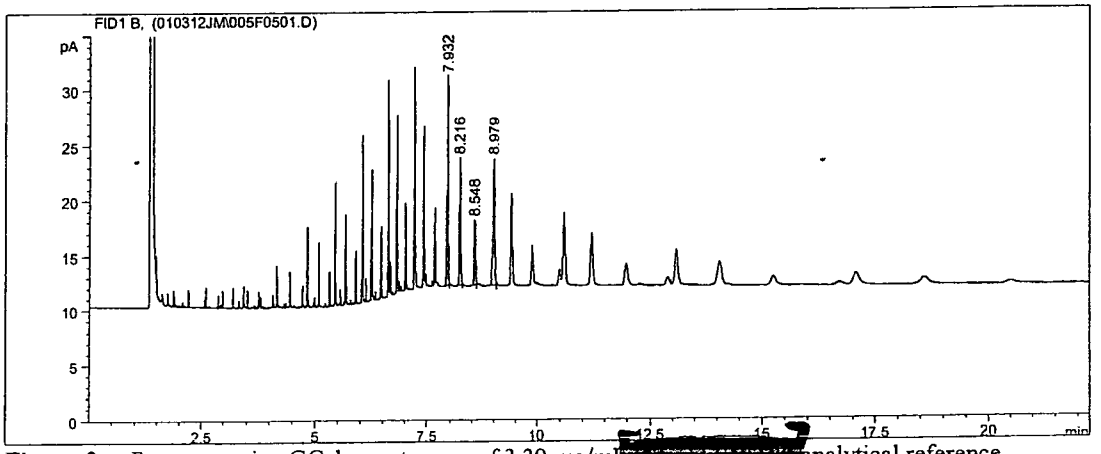


**Figure 2c:** Representative GCchromatogram of 3.29 mg/mL [redacted] analytical reference solution.

# Table 1

## Summary of Dosing Solution Analyses

| Sample Type | | Dosing Concentrations and Stability of [redacted] (ng/mL) | | | |
|---|---|---|---|---|---|
| 2/26/01 | Nominal: | 5.0 | 10.0 | 40.0 | 70.0 |
| Concentration Verification | | | | | |
| | | 4.37 | 8.40[b] | 34.6[b] | 61.0 |
| | | (87.4)[a] | (84.0) | (86.5) | (87.1) |
| | | 4.23[b] | 8.39 | 32.0 | 61.7[b] |
| | | (84.6) | (83.9) | (80.0) | (88.1) |
| **Average Measured Conc.[c]** | | **4.30** | **8.39** | **33.3** | **61.4** |
| **Average Percent Nominal[c]** | | **(86.0)** | **(83.9)** | **(83.3)** | **(87.7)** |
| **Standard Deviation[c]** | | **± 0.1** | **± 0.01** | **± 1.8** | **± 0.5** |
| **Coefficient of Variation[c]** | | **2%** | **1%** | **5%** | **1%** |
| Stability | | 4.18[c] | 8.05[c] | 31.6[c] | 59.2[c] |
| | | (83.7) | (80.5) | (79.0) | (84.5) |
| | | 5.02[d] | 9.31[d] | 37.8[d] | 65.5[d] |
| | | (100.4) | (93.1) | (94.5) | (93.5) |
| 3/7/01 | | | | | |
| Concentration Verification | | | | | |
| | | 4.72 | 9.36 | 40.2 | 63.2 |
| | | (94.4)[a] | (93.6) | (100.5) | (90.2) |
| | | 4.54 | 8.75 | 38.9 | 61.7 |
| | | (90.8) | (87.5) | (97.3) | (88.1) |
| **Average Measured Conc.[c]** | | **4.63** | **9.05** | **39.6** | **62.4** |
| **Average Percent Nominal[c]** | | **(92.6)** | **(90.5)** | **(98.9)** | **(89.1)** |
| **Standard Deviation[c]** | | **± 0.1** | **± 0.4** | **± 0.9** | **± 1.1** |
| **Coefficient of Variation[c]** | | **3%** | **5%** | **2%** | **2%** |
| Stability | | 5.06[e] | 9.70[e] | 42.1[e] | 84.2[e] |
| | | (101.2) | (97.0) | (105.2) | (120.3) |
| | | 5.37[f] | 9.72[f] | 41.3[f] | 69.7[f] |
| | | (107.4) | (97.2) | (103.3) | (99.6) |

a Numbers in parentheses are the respective percent of nominal values.
b Mean result of all analyses (n=2).
c Mean, S.D. and C.V. for duplicate samples calculated to verify uniformity of mixture.
d Samples held at room temperature for 5 hours.
e Samples held refrigerated for 4 days and sampled.
f Samples held refrigerated for 4 days followed by 5 hours at room temperature.