Haskell Laboratory for Toxicology
and Industrial Medicine

January 4, 2002

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

cc: L. A. Malley

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ANALYSIS FOR H-24516 IN DOSING SUSPENSIONS

Medical Research Project Number:	[redacted]
Haskell Sample Number:	24516
Analytical Reference:	24516
Analytical Report Number:	HA-2001-045
Service Code:	
Study Number:	[redacted]
Notebook References:	

Attached is the analytical report to for the study identified above.

[redacted] Pilot Developmental Toxicity Study in Rats

ANALYSIS FOR [redacted] IN DOSING SUSPENSIONS

Medical Research Project Number: [redacted]
Haskell Sample Number: 24516
Analytical Report Number: HA-2001-045

SUMMARY

Dosing suspensions at concentrations of 12.5, 25.0, 50.0 and 100.0 mg/mL of H-24516 were collected for homogeneity/concentration verification and 5 hour room temperature stability analysis on January 16, 2001. Subsequently on January 19, 2001, the refrigerated stability samples along with a 5-hour room temperature sample for the 100 mg/mL level from the same preparation were submitted. Only the 100.0 mg/mL level was sampled for the refrigerated stability because the other levels in this study were within the range of a previous study (MR[redacted]SC[redacted]) in which the mixing and refrigerated stability had been established. In addition, 0 mg/mL (control) samples were submitted for analysis with each set of samples.

Dosing suspensions at concentrations of 12.5, 25.0, and 50.0 mg/mL of H-24516 were submitted for concentration verification analysis on January 27, 2001. On the same day, dosing suspensions at concentrations of 100.0 mg/mL of H-24516 were submitted for homogeneity/concentration verification analysis to confirm proper mixing at this level. In addition, the 0 mg/mL (control) sample was submitted for analysis.

H-24516 as used in this report refers to the active ingredient (a.i.) in [redacted] New Process.

Concentrations of H-24516 in separate dosing suspensions were measured by gas chromatography (GC).

The suspension vehicle for the study was 0.5% aqueous methylcellulose.

Results from analysis of the dosing suspensions collected on January 16, 2001 indicated that test substance was at the expected concentrations (± 7.5%) for all samples, homogeneously mixed in the vehicle except for the 100 mg/mL level (C.V. = 12%), and stable in the vehicle for 5 hours at room temperature. The 100 mg/mL was not sufficiently mixed before sampling for the analytical work. This was substantiated by the results for the refrigerated and room temperature stability analysis from this preparation.

Results for samples collected January 19, 2001 for the 100 mg/mL indicated that the test substance was at the targeted level (86.7% of nominal) and stable in the vehicle when held refrigerated for 4 days and followed by 5 hours at room temperature.

Samples collected January 27, 2001 for the 100 mg/mL indicated that the level was

homogeneously mixed (C.V. = 4%), and at the expected concentration (± 7.3%). Results for the other concentrations indicated that they were uniformly mixed (C.V.'s of 1% and 2%, respectively) and at the targeted levels (± 19%).

H-24516 was not detected in any of the 0 mg/mL (control) sample in the study.

SAMPLE SUBMITTAL

Samples containing H-24516 at the concentrations of 0, 12.5, 25.0, 50.0 and 100.0 mg/mL were collected on January 16, 2001. These samples were analyzed to determine homogeneity/concentration verification and 5-hour room temperature stability. Samples from the same preparation at the concentrations of 0, 100.0 mg/mL were collected on January 19, 2001. These samples were analyzed to determine 4-day refrigerated stability along with 5-hour room temperature stability. The 4-day refrigerated stability for the remainder of the levels in this study had been established in a previous study (MR[redacted] SC[redacted] with H-24516.

Samples containing H-24516 at the concentrations of 0, 12.5, 25.0, 50.0 and 100.0 mg/mL were collected on January 27, 2001. The 100.0 mg/mL samples were analyzed to determine homogeneity/concentration verification while the remaining levels were analyzed for concentration verification.

All dosing suspension samples were collected on the same day the suspensions were prepared or at the prescribed protocol time for analysis. They were analyzed when received or were frozen until analyzed.

The suspension vehicle was 0.5% aqueous methylcellulose.

METHODS

1. Analytical Methods

a. Dosing Suspension Treatment

Each dosing sample was diluted to 100 mL with methanol and sonicated to dissolve the H-24516 in the suspension. The dosing samples were further diluted with the 0 mg/mL sample (initial dilution) to an expected concentration of approximately 0.09 mg/mL (a.i.) prior to analysis. An internal standard (refer to Calibration and Quantitation Section) at an equivalent amount was added to each sample before the final analysis dilution.

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

b. Chromatographic Conditions

Instrument:	Hewlett-Packard Model 6890 GC
Column:	J & W, DB-1701, 30 m, 0.32 mm ID, 1 um film thickness
Injector:	Split, 250°C
Detector:	FID; 250°C
Carrier Gas:	Helium (2.1 mL/min)
Split vent:	22.3 mL/min
Injection Volume:	2 microliter
Oven Program:	Gradient
Initial Temperature:	100°C
Initial Time	1.00 min.
Level 1 Rate:	20.0°C/min.
Level 1 Temperature:	260°C
Level 1 Time:	0.00 min.
Total run time:	9.00 min.

c. Calibration and Quantitation

A separate sample of H-24516 (-3) was obtained to use as the analytical reference standard (94.7% pure). A stock solution was prepared in methanol. This stock solution was sonicated to assure that all material was in solution. Before analysis, appropriate aliquots of the stock were diluted with methanol to make calibration standards that bracketed the target concentration of the diluted dosing samples. A stock solution of an internal standard (1H, 1H, 2H, 2H-perfluoro-9-methyldecan-1-0l, 98% pure) was prepared in methanol and added to each calibration standard and test sample to give an equivalent final concentration in all dilutions. The ratio of the peak heights for internal standard and H-24516 at three retention times (3.5, 4.17, and 4.77 minutes) from replicate GC analysis of these solutions were used to construct a calibration curves by least squares regression (see Figure 1a, 1b, and 1c for a representative curves). Measured concentrations for the dosing samples were determined by applying the peak height ratios from replicate injections of each sample to the respective calibration curve.

Test substance homogeneity in the dosing suspensions was evaluated by calculating the coefficient of variation (C.V. = standard deviation/mean x 100) of the measured concentrations in the top, middle, and bottom (T, M, and B) samples or the mean of duplicate samples for each concentration. A coefficient of variation of less than 10% is the standard criterion at Haskell Laboratory for acceptable distribution of the test substance throughout the dosing suspension. The mean result of the top, middle, and bottom homogeneity samples or of the duplicate concentration verification 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 top, middle, and bottom homogeneity samples or of the duplicate concentration verification samples (0-day room temperature) as the baseline for comparing the corresponding room temperature and refrigerated results.

ANALYTICAL RESULTS

A. Chromatography

H-24516 eluted from the GC column as resolved peaks with retention times from 2.9 minutes to approximately 6.0 minutes. For the purpose of quantitation, resolved peaks at the retention times of approximately 3.5, 4.17, and 4.77 minutes were used. The internal standard [redacted] eluted as a resolved peak at approximately 3.99 minutes. Representative GC chromatograms are shown in Figures 2(a - c). Test substance was not detected in the 0 mg/mL control.

B. Homogeneity/Concentration Verification and Stability Samples

Analytical results from dosing suspensions collected on January 16, 2001 and analyzed for homogeneity/concentration verification and stability are shown in Table I and Summary Table 1.

The following table summarizes the results for homogeneity/concentration verification and stability analyses.

Preparation Date	Nominal mg/mL	Measured T,M,B[a] mg/mL	Mean (T,M,B) % Nominal	C.V. (%)	Stability[b] % Nominal
16-Jan-2001	0	ND[c]	---	---	---
	12.5	12.8, 12.7, 12.6	101.8	1	98.7
	25	23.3, 24.6, 25.0	97.2	4	94.7
	50	53.8, 50.5, 52.7	104.6	3	100.2
	100	93.5, 74.8, 78.9	82.4	12	83.5

a Mean results for the analysis of the top (T), middle (M) and bottom (B) samples.
b Samples held 5 hours at room temperature.
c Denotes none detected.

The data for samples collected on January 16, 2001 indicates that the test substance was homogeneously mixed in the vehicle except for the 100.0 mg/mL level (C.V. = 12). The test substance was at the targeted concentration in the samples (± 17.6% of nominal) and was stable in the vehicle when held 5 hours at room temperature.

Test substance was not found in the 0 mg/mL samples.

C. Stability Study

Analytical results from dosing suspensions collected on January 19, 2001 and analyzed for concentration verification and stability (4 days refrigerated followed by 5 hours room temperature) are shown in Table II and Summary Table 1.

The following table summarizes the results for homogeneity/concentration verification (January 16, 2001) and stability analyses (January 19, 2001).

Preparation Date Sample Type	Nominal mg/mL	Measured mg/mL	Mean % Nominal	C.V. (%)	Stability % Nominal
16-Jan-2001					
Homogeneity[a]	0	ND[b]	---	---	---
	100	93.5, 74.8, 78.9	82.4	12	83.5[c]
19-Jan-2001					
Stability					
4-Day Refrigerated	100	86.7[d]	86.7	---	94.3[e]

a Mean results for the analysis of the top (T), middle (M) and bottom (B) samples.
b Denotes none detected.
c Samples held 5 hours at room temperature.
d Mean result of single sample for concentration verification/4-day refrigerated.
e Mean result of single sample for 4 day-refrigerated/5 hour room temperature stability.

The data for samples collected on January 16, 2001 indicates that the test substance was not homogeneously mixed in the vehicle (C.V. = 12%) but was at the targeted concentration in the samples (± 13% of nominal). Comparison of the data for the stability samples to the mean concentration indicates that the test substance is stable in the vehicle when held 4 days refrigerated and followed by 5 hours at room temperature.

Test substance was not found in the 0 mg/mL samples.

The following table is included for a reference in this study to the refrigerated stability for the concentration range. It summarizes the results for MR[redacted]SC[redacted] homogeneity/concentration verification (January 8, 2001 and January 15, 2001) and the 4-day refrigerated stability analyses followed by 5 hours room temperature.

Preparation Date Sample Type	Nominal mg/mL	Measured mg/mL	Mean % Nominal	C.V. (%)	Stability % Nominal
8-Jan-2001					
Homogeneity [a]	0	ND[b]	---	---	---
	5.0	4.53, 4.25, 4.39	87.8	3	92.0[c]
	20	19.9, 17.7, 18.6	93.5	6	86.0[c]
	50	53.4, 52.6, 54.0	106.8	1	96.6[c]
Stability					
4-Day Refrigerated[d]	5.0	4.81	96.1	---	97.1
4-Day Refrigerated[d]	20	19.6	97.8	---	88.2
4-Day Refrigerated[d]	50	48.9	97.8	---	95.7
15-Jan-2001					
Homogeneity [a]	0	ND[b]	---	---	---
	3.33	2.88, 2.54, 2.67	81.1	6	79.9
Stability					
4-Day Refrigerated[f]	3.33	2.94, 2.88	87.4	1	82.3

a Mean results for the analysis of the top (T), middle (M) and bottom (B) samples.
b Denotes none detected.
c Samples held 5 hours at room temperature.
d Mean result of single sample for 4-day refrigerated/concentration verification and a single sample for 4-day refrigerated/5 hour room temperature stability.
f Mean results of duplicate samples for 4-day refrigerated/concentration verification and a single sample for 4-day refrigerated/5 hour room temperature stability.

The data from MR[redacted]SC[redacted] samples along with the data for the 100 mg/mL samples in this study indicate that the test substance is stable in the vehicle when held 4 days refrigerated and followed by 5 hours at room temperature for the concentration range of the study.

D. Concentration Verification/Homogeneity

Analytical results from dosing suspensions collected on January 27, 2001 and analyzed for homogeneity and/or concentration verification are shown in Table III and Summary Table 1.

The following table summarizes the results for homogeneity and/or concentration verification analyses.

Preparation Date	Nominal mg/mL	Measured[a] mg/mL	Mean (T,M,B) % Nominal	C.V. (%)
27-Jan-2001	0	ND[b]	---	---
	12.5	10.7, 10.6	85.3	0.2[c]
	25.0	20.3, 20.1[c]	80.9	1[c]
	50.0	46.9, 46.6[c]	93.5	1[c]
	100.0	91.6, 97.1, 89.5	92.7	4

a Mean results for the analysis of the top (T), middle (M) and bottom (B) samples or of the duplicate samples.
b Denotes none detected.
c C.V. for the duplicate samples used to confirm uniformity of mixture.

The data for samples collected on January 27, 2001 indicate that the test substance was homogeneously mixed in the vehicle at all levels. The test substance was at the targeted concentration in the samples (± 20% of nominal).

Test substance was not found in the 0 mg/mL samples.

E. Conclusions

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

ACKNOWLEDGMENTS

Analysis of dosing samples by Sheila A. Riley (Chemistry Associate).

SIGNATURES

Report by: Janet C. Maslanka — 4-JAN-2002
Janet C. Maslanka
Staff Scientist
Date

Date issued: 4-JAN-2002

Table I. Homogeneity and Stability of H-24516 in Dosing Suspensions

Sample Type	mg/mL H-24516 Nominal	Measured	Percent Nominal
16-Jan-2001			
Homogeneity			
CONTROL	0.00	ND(A)	----
TOP	12.5	12.8	102.4
MIDDLE	12.5	12.7	101.9
BOTTOM	12.5	12.6	101.1
	Mean(B):	*12.7±0.1*	*(101.8%)*
		C.V. 1%	
TOP	25.0	23.3	93.3
MIDDLE	25.0	24.6	98.4
BOTTOM	25.0	25.0	100.0
	Mean(B):	*24.3±0.9*	*(97.2%)*
		C.V. 4%	
TOP	50.0	53.8	107.5
MIDDLE	50.0	50.5	101.0
BOTTOM	50.0	52.7	105.3
	Mean(B):	*52.3±1.7*	*(104.6%)*
		C.V. 3%	
TOP	100.0	93.5	93.5
MIDDLE	100.0	74.8(C)	74.8
BOTTOM	100.0	78.9(C)	78.9
	Mean(B):	*82.4±9.8*	*(82.4%)*
		C.V. 12%	
Stability(D)			
	12.5	12.3	98.7
	25.0	23.7	94.7
	50.0	50.1	100.2
	100.0	83.5	83.5

(A) Denotes not detected.
(B) The average measured concentration, average percent of nominal (in parentheses), standard deviation, and coefficient of variation of top, middle, and bottom are based on measured concentration for each level.
(C) Mean result of the original analysis and duplicate re-analysis of original sample reported.
(D) Stability samples held 5 hours a room temperature.

Table II. Stability Study of H-24516 in Dosing Suspensions

Preparation Date / Sample Type	mg/mL H-24516 Nominal	mg/mL H-24516 Measured	Percent Nominal
15-Jan-2001(A)			
0-DAY ROOM TEMPERATURE(B)	3.33	2.70	81.1
4-DAY REFRIGERATED	3.33	2.94	88.3
	3.33	2.88	86.5
		Mean(C): 2.91±0.04	*(87.4%)*
		C.V. 1%	
4-DAY REFRIGERATED/5 HOUR RT	3.33	2.74	82.3
8-Jan-2001(A)			
0-DAY ROOM TEMPERATURE(B)	5.0	4.39	87.8
4-DAY REFRIGERATED	5.0	4.81	96.1
4-DAY REFRIGERATED/5 HOUR RT	5.0	4.85	97.1
0-DAY ROOM TEMPERATURE(B)	20.0	18.7	93.5
4-DAY REFRIGERATED	20.0	19.6	97.8
4-DAY REFRIGERATED/5 HOUR RT	20.0	17.6	88.2
0-DAY ROOM TEMPERATURE(B)	50.0	53.4	106.8
4-DAY REFRIGERATED	50.0	48.9	97.8
4-DAY REFRIGERATED/5 HOUR RT	50.0	47.9	95.7
16-Jan-2001(D)			
0-DAY ROOM TEMPERATURE(B)	100.0	82.4	82.4
4-DAY REFRIGERATED	100.0	86.7	86.7
4-DAY REFRIGERATED/5 HOUR RT	100.0	94.3	94.3

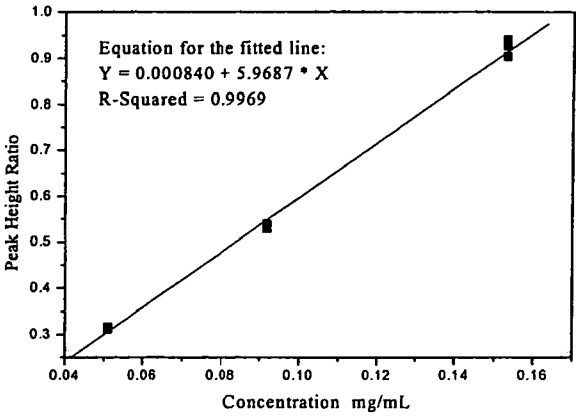
(A) Samples from [redacted]
(B) The average measured concentration of top, middle, and bottom for the homogeneity samples (0-Day Room Temperature) used as baseline for stability comparison.
(C) Reported result is the mean of the re-analysis of the duplicate original samples. Original analysis indicated aliquot error and will not be reported.
(D) Samples from [redacted]

Table III. Homogeneity/Concentration Verification of H-24516 in Dosing Suspensions

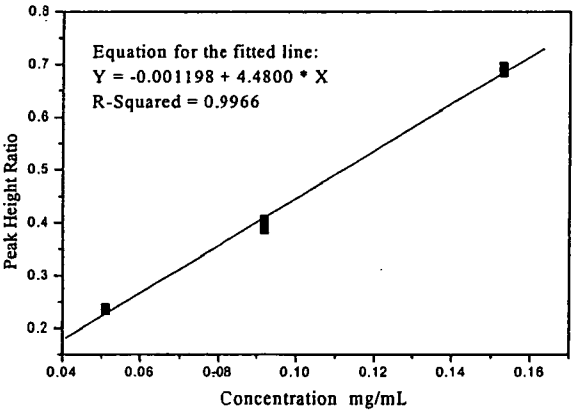
Sample Type	mg/mL H-24516 Nominal	Measured	Percent Nominal
27-Jan-2001			
Concentration Verification			
CONTROL	0.00	ND(A)	----
#1	12.5	10.7	85.3
#2	12.5	10.6	85.1
		Mean(B): 10.7±0.02	***(85.2%)***
		C.V. 0.2 %	
#1	25.0	20.3	81.3
#2	25.0	20.1	80.5
		Mean(B): 20.2±0.14	***(80.9%)***
		C.V. 1%	
#1	50.0	46.9	93.9
#2	50.0	46.6	93.1
		Mean(B): 46.8±0.26	***(93.5%)***
		C.V. 1%	
Homogeneity			
TOP	100.0	91.6	91.6
MIDDLE	100.0	97.1	97.1
BOTTOM	100.0	89.5	89.5
		Mean(C): 92.7±3.9	***(92.7%)***
		C.V. 4%	

(A) Denotes not detected.
(B) The average measured concentration, average percent of nominal (in parentheses), standard deviation, and coefficient of variation of duplicate samples are based on measured concentration for each level.
(C) The average measured concentration, average percent of nominal (in parentheses), standard deviation, and coefficient of variation of top, middle, and bottom are based on measured concentration for each level.

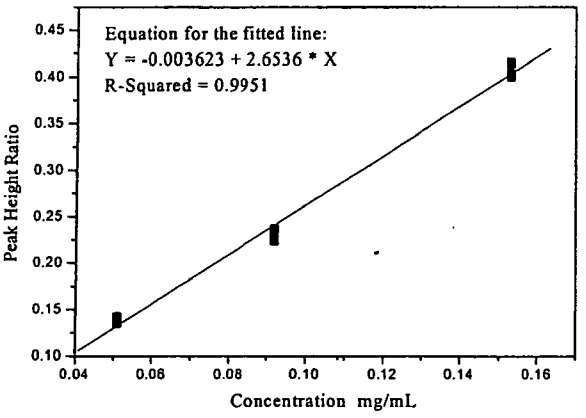
Figure 1
Representative Analytical Calibration Curve



(a)



(b)



(c)

Figure 1: Calibration curve showing linear fit (line) to replicate peak height ratio measurements (squares) for calibration solutions of H-24516 diluted over a concentration range of 0.0510 to 0.1531 mg/mL. [(a) Retention time = 3.5 minutes, (b) Retention = 4.17 minutes, (c) Retention time = 4.77 minutes.]

Figure 2
Representative High Performance Liquid Chromatography Chromatograms

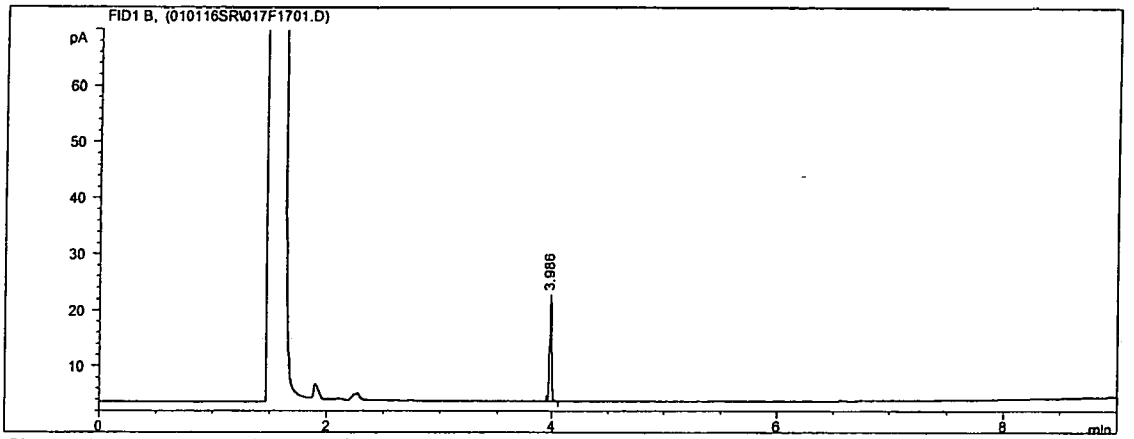


Figure 2a: Representative GC chromatogram of 0 mg/mL (control) sample with internal standard [redacted] added. Retention time for H-24516 is approximately 3.5, 4.1, 4.7 minutes. Retention time for internal standard is approximately 3.9 minutes.

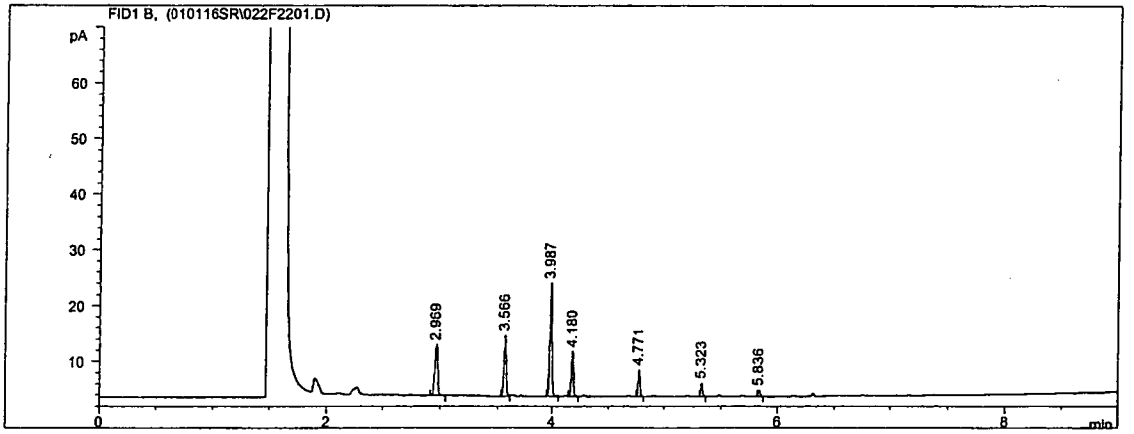


Figure 2b: Representative GC chromatogram of 12.5 mg/mL H-24516 dosing solution diluted to a nominal concentration of 0.09 mg/mL. The measured concentration of the representative solution is 12.8 mg/mL.

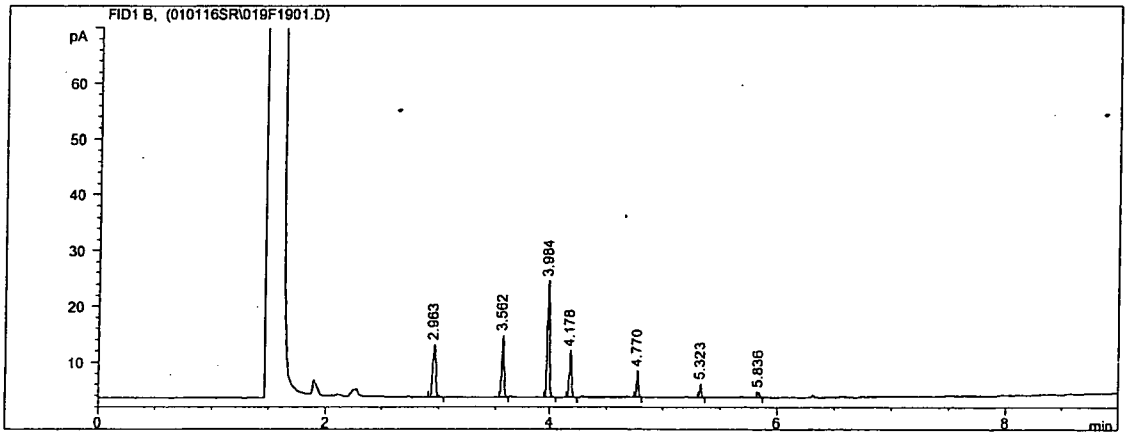


Figure 2c: Representative GC chromatogram of 0.0919 mg/mL H-24516 analytical reference solution.

TABLE 1
SUMMARY OF DOSING ANALYSES

	Dosing Concentration of H-24516 (mg/mL)			
Nominal:	12.5	25.0	50.0	100.0
Homogeneity Samples				
1-Jan-2001				
Top	12.8 (102.4)[a]	23.3 (93.3)	53.8 (107.5)	93.5 (93.5)
Middle	12.7 (101.9)	24.6 (98.4)	50.5 (101.0)	74.8 (74.8)
Bottom	12.6 (101.1)	25.0 (100.0)	52.7 (105.3)	78.9 (78.9)
Average Measured Conc.[b]	**12.7**	**24.3**	**52.3**	**82.4**
Average Percent Nominal	**(101.8)**	**(97.2)**	**(104.6)**	**(100.0)**
Standard Deviation[b]	**± 0.1**	**± 0.9**	**± 1.7**	**± 9.8**
Coefficient of Variation[b]	**1%**	**4%**	**3%**	**12%**
Stability Samples				
5-Hour Room Temperature	12.3 (98.7)	23.7 (94.7)	50.1 (100.2)	83.5 (83.5)
27-Jan-2001				
Concentration Verification[c]				
Top	10.7 (85.3)	20.3 (81.3)	46.9 (93.9)	91.6 (91.6)
Middle	10.6 (85.1)	20.1 (80.5)	46.6 (93.1)	97.1 (97.1)
Bottom	---[c]	---[c]	---[c]	89.5 (89.5)
Average Measured Conc.[b]	**10.7**	**20.2**	**46.8**	**92.7**
Average Percent Nominal	**(85.2)**	**(80.9)**	**(93.5)**	**(92.7)**
Standard Deviation[b]	**± 0.02**	**± 0.14**	**± 0.26**	**± 3.9**
Coefficient of Variation[b]	**0.2%**	**1%**	**1%**	**4%**

[a] Numbers in parentheses are the respective percent of nominal values.
[b] Statistics based on the average measured concentration (ppm) of the top, middle and bottom of each dosing level or duplicate samples.
[c] Samples not required for study.

TABLE 1 (continued)
SUMMARY OF DOSING ANALYSES

	Dosing Concentration of H-24516 (mg/mL)				
Stability Study Nominal:	3.33 [a]	5.0 [b]	20.0 [b]	50.0 [b]	100.0 [c]
Homogeneity Samples					
Top	2.88 (86.5) [d]	4.53 (90.6)	19.9 (99.5)	53.4 (106.8)	93.5 (93.5)
Middle	2.54 (76.3)	4.25 (85.0)	17.7 (88.5)	52.6 (105.2)	74.8 (74.8)
Bottom	2.67 (80.2)	4.39 (87.8)	18.6 (93.0)	54.0 (108.0)	78.9 (78.9)
Average Measured Conc. [e]	**2.70**	**4.39**	**18.7**	**53.4**	**82.4**
Average Percent Nominal	**(81.1)**	**(87.8)**	**(93.5)**	**(106.8)**	**(82.4)**
Standard Deviation [e]	**± 0.2**	**± 0.14**	**± 1.1**	**± 0.7**	**± 9.8**
Coefficient of Variation [e]	**6%**	**3%**	**6%**	**1%**	**12%**
Stability Samples					
0-Day Room Temperature [f]	2.70 (81.1)	4.39 (87.8)	18.7 (93.5)	53.4 (106.8)	82.4 (82.4)
4-Day Refrigerated	2.91 (87.4)	4.81 (96.1)	19.6 (97.8)	48.9 (97.8)	86.7 (86.7)
4-Day Refrigerated/5 hr.	2.74 (82.3)	4.85 (97.1)	17.6 (88.2)	47.9 (95.7)	94.3 (94.3)

[a] Samples prepared January 15, 2001 for [redacted]
[b] Samples prepared January 8, 2001 for [redacted]
[c] Samples prepared January 27, 2001 for [redacted]
[d] Numbers in parentheses are the respective percent of nominal values.
[e] Statistics based on the average measured concentration (ppm) of the top middle and bottom of each dosing level.
[f] The mean measured values from analysis of homogeneity samples (considered fresh/0-day room temperature samples) were used as baselines for comparison with the respective stability samples.