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Haskell Laboratory for Toxicology
and Industrial Medicine

March 13, 2003

TO: MR. [redacted] Repeated-Dose Oral Toxicity Gavage Range Finder Study in Rats

cc: G. A. Ladics

FROM: J. C. Maslanka
Analytical Chemist

ANALYSIS FOR H-24691 IN DOSING SUSPENSIONS

MIXING AND STABILITY STUDY

REVISION No. 1

Medical Research Project Number:	[redacted]
Haskell Sample Number:	24691
Analytical Reference:	24691
Analytical Report Number:	HA-2001-037
Service Code:	[redacted]
Study Number:	[redacted]
Notebook References:	

Attached is the analytical report to satisfy protocol requirements for toxicology studies with [redacted] but may be used for other purposes.

Company Sanitized. Does not contain TSCA CBI

SAMPLE SUBMITTAL

Samples containing H-24691 at the concentrations of 0, 1.0, 5.0, and, 25.0 mg/mL were collected on Test Day 4. These samples were analyzed to determine homogeneity/concentration verification and 5-room temperature stability. Samples from the same preparation were collected on Test Day 11 after 7 days of refrigeration. These samples were analyzed to determine homogeneity (1.0 mg/mL level only), concentration verification and refrigerated stability along with 5-hour room temperature stability after refrigeration.

All dosing suspension samples were collected on the same day the suspensions were prepared or at the established stability time for analysis. They were analyzed when received.

The suspension vehicle was 0.5% methylcellulose.

METHODS

1. Analytical Methods

a. Dosing Suspension Treatment

Each dosing sample (3 mL) was diluted to 100 mL with methanol and mixed to dissolve the H-24691 in the suspension. The dosing samples were analyzed without further dilution or were further diluted with the 0 mg/mL sample (initial dilution) to an expected concentration of approximately 0.03mg/mL (a.i.) prior to analysis. Before all final dilutions, the internal standard diluted solution (refer to Calibration and Quantitation Section) was added to each test sample to give a final concentration of approximately 0.04 mg/mL. Each sample (final dilution) had an equivalent amount matrix (methylcellulose in methanol) when analyzed.

Samples submitted for analysis were analyzed the day the suspensions were received 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-24691 was obtained to use as the analytical reference standard. 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 the 0 mg/mL sample (initial dilution) to make calibration standards, which bracketed the target concentration of the diluted dosing samples. A stock solution of internal standard (1H, 1H, 2H, 2H-perfluoro-9-methyldecan-1-ol, 98% pure) was prepared in methanol and added to each calibration standard and test sample to give a final concentration of 0.04 mg/mL. The ratio of the internal standard and H-24691 peak heights from replicate GC analysis of these solutions were used to construct a calibration curve by least squares regression (see Figure 1 for a representative curve). Measured concentrations for the dosing samples were determined by applying the peak height ratios from replicate injections of each sample to the calibration curve.

ANALYTICAL RESULTS

A. Chromatography

H-24691 eluted from the GC column as resolved peak with a retention time of approximately 3.5 minutes. The internal standard eluted from the GC column as a resolved peak with a retention time of approximately 3.9 minutes. Representative GC chromatograms are shown in Figures 2(a - c). Test substance was not detected in the 0 mg/mL control.

B. Mixing and Stability Study

Analytical results from dosing suspensions collected on Test Day 4 and Test Day 11 and analyzed for homogeneity and/or concentration verification and stability are shown in Table I and Summary Table 1.

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

Test Day Sample Type	Nominal mg/mL	Measured T,M,B[a] mg/mL	Mean (T,M,B) % Nominal	C.V. (%)	Stability[b] % Nominal
Test Day 4					
Homogeneity	0	ND[c]	---	---	---
	1.0	0.944, 0.884, 0.836[d]	88.8	6	85.4[e]
	5.0	4.43, 4.37, 4.72[d]	90.2	4	95.0
	25	22.2, 22.7, 24.2	92.0	5	96.0
Test Day 11					
Stability					
7 Day Refrigerated[f]	0	ND[c]	---	---	---
	1.0	0.900, 0.864, 0.859	87.4	3	90.4[g]
	5.0	4.46, 4.44	89.0	0	84.2[g]
	25	24.2, 23.9	96.4	1	84.8[g]

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.
d Mean result of all analyses reported.
e Mean result of all analyses reported except for one duplicate repeat sample which was low due to aliquot error.
f Duplicate concentration verification samples analyzed except for the 1.0 mg/mL level (homogeneity).
g Mean result of duplicate re-sampling from the original diluted sample. Original analysis was lower than expected due to aliquot error and not reported.

The data for samples collected on Test Day 4 indicates that the test substance was homogeneously mixed in the vehicle at all levels (C.V.'s = 6, 4 and 5, respectively). The test substance was at the targeted concentration in the samples (± 11.2% of nominal) and was stable in the vehicle when held 5 hours at room temperature.

The data for samples collected on Test Day 11 after 7 days of refrigeration indicates that the test substance was homogeneously re-suspended in the vehicle at all levels (C.V.'s = 3, 0 and 1, respectively). The test substance was at the targeted concentration (± 12.6% of nominal) and stable in the vehicle when held refrigerated for 7 days followed by 5 hours at room temperature. The 5-hour sample for the 25 mg/mL level (84.8% of nominal) appeared to be less stable than expected but this can be attributed to sampling and analytical variability and not stability of the test material in the vehicle.

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

C. Conclusions

Data from the analysis of the samples during the mixing study indicate that the test substance could be mixed homogeneously, was at the targeted levels and stable under the conditions necessary for mixing the dosing suspensions twice weekly for any study within the range of the concentrations tested. Test substance was not found in the 0 mg/mL samples.

ACKNOWLEGEMENTS

Sample analysis by Sheila A. Riley, (Chemistry Associate).

SIGNATURES

Report by: Janet C. Maslanka — 13-Mar-2003
Janet C. Maslanka, Chemist — Day/Mo/Yr

Date issued: 13-Mar-2003

Table I. Homogeneity and Stability of H-24691 in Dosing Suspensions

Sample Type	mg/mL H-24691 Nominal	Measured	Percent Nominal
Test Day 4			
Homogeneity			
CONTROL	0.00	ND(A)	----
TOP	1.0	0.944	94.4
MIDDLE	1.0	0.884	88.4
BOTTOM	1.0-O	0.809	80.9
	1.0-1	0.860	86.0
	1.0-2	0.840	84.0
	mean(B):	*0.836±0.03*	*(83.6%)*
	Mean(C):	*0.888±0.05*	*(88.8%)*
		C.V. 6%	
TOP	5.0	4.43	88.6
MIDDLE	5.0	4.37	87.4
BOTTOM	5.0-O	4.16	83.2
	5.0-1	4.72	94.4
	5.0-2	5.27	105.4
	mean(B):	*4.72±0.56*	*(94.4%)*
	Mean(C):	*4.51±0.19*	*(90.2%)*
		C.V. 4%	
TOP	25.0	22.2	88.8
MIDDLE	25.0	22.7	90.8
BOTTOM	25.0	24.2	96.8
	Mean(C):	*23.0±1.0*	*(92.0%)*
		C.V. 5%	
Stability(D)			
	1.0-O	0.818	81.8
	1.0-1	0.883	88.3
	1.0-2A	0.893	89.3
	1.0-2B	0.822	82.2
	mean(E):	*0.854±0.04*	*(85.4%)*
	5.0	4.75	95.0
	25.0	24.0	96.0

(A) Denotes not detected.
(B) Mean result of the original aliquot (-O) and duplicate aliquots from the original diluted sample (-1, -2).
(C) The average measured concentration, average percent of nominal (in parentheses), standard deviation, and coefficient of variation of top, middle, and bottom (mean result, n=3) except for 25 mg/mL level bottom (n=1).
(D) Stability samples held 5 hours a room temperature.
(E) Mean result of original (-O) aliquot, one duplicate (-1) aliquot and duplicate re-analysis of the second aliquot (-2A, -2b).

Table I. Homogeneity and Stability of H-24691 in Dosing Suspensions (continued)

Test Day / Sample Type	mg/mL H-24691 Nominal	Measured	Percent Nominal
Test Day 11			
Homogeneity/Stability			
CONTROL	0.00	ND(A)	----
TOP	1.0	0.900	90.0
MIDDLE	1.0	0.864	86.4
BOTTOM	1.0	0.859	85.9
		Mean(B): 0.874±0.02	***(87.4%)***
		C.V. 3%	
Concentration Verification/Stability			
#1	5.0	4.46	89.2
#2	5.0	4.44	88.8
		Mean(C): 4.45±0.01	***(89.0%)***
		C.V. 0%	
#1	25.0	24.2	96.8
#2	25.0	23.9	95.6
		Mean(C): 24.1±0.21	***(96.4%)***
		C.V. 1%	
Stability			
7-DAY REFRIGERATED/5 HOUR RT	1.0	0.955	95.5
	1.0	0.852	85.2
		Mean(D): 0.904±0.07	***(90.4%)***
		C.V. 8%	
7-DAY REFRIGERATED/5 HOUR RT	5.0	4.18	83.6
	5.0	4.23	84.6
		Mean(D): 4.21±0.04	***(84.2%)***
		C.V. 1%	
7-DAY REFRIGERATED/5 HOUR RT	25.0	20.5	82.0
	25.0	21.8	87.2
		Mean(D): 21.2±0.92	***(84.8%)***
		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 top, middle, and bottom.
(C) The average measured concentration, average percent of nominal (in parentheses), standard deviation, and coefficient of variation of duplicate samples.
(D) The average measured concentration, average percent of nominal (in parentheses), standard deviation, and coefficient of variation of duplicate samples. Mean result of duplicate re-analysis of the original sample. Original sample analysis was lower than expected not reported.

Figure 1
Representative Analytical Calibration Curve

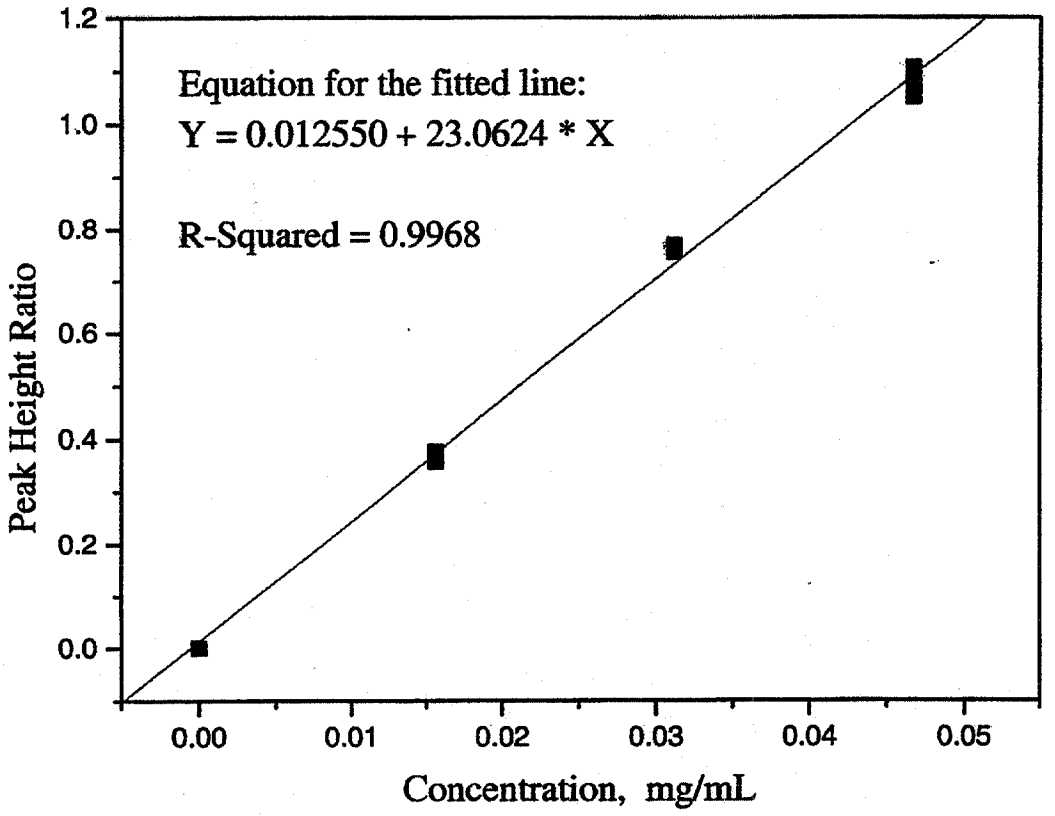

Figure 1: Calibration curve showing linear fit (line) to replicate peak height ratio measurements (squares) for calibration solutions of H-24691 diluted over a concentration range of 0.0156 to 0.0468 mg/mL. The 0 mg/mL control diluted sample was used as the zero.

Figure 2
Representative Gas Chromatography Chromatograms

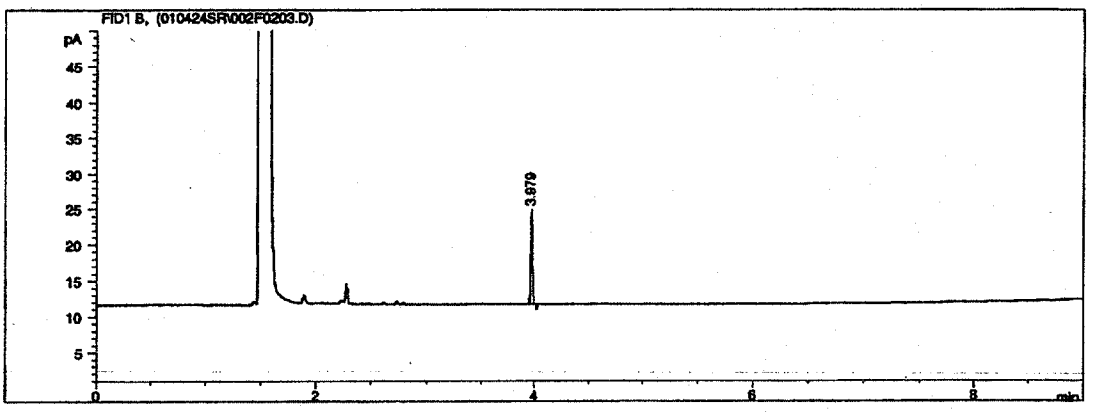

Figure 2a: Representative GC chromatogram of 0 mg/mL (control) sample with internal standard (1H,1H,2H,2H-perfluoro-9-methyldecan-1-ol) added. Retention time for H-24691 is approximately 3.5 minutes. Retention time for internal standard is approximately 3.9 minutes.

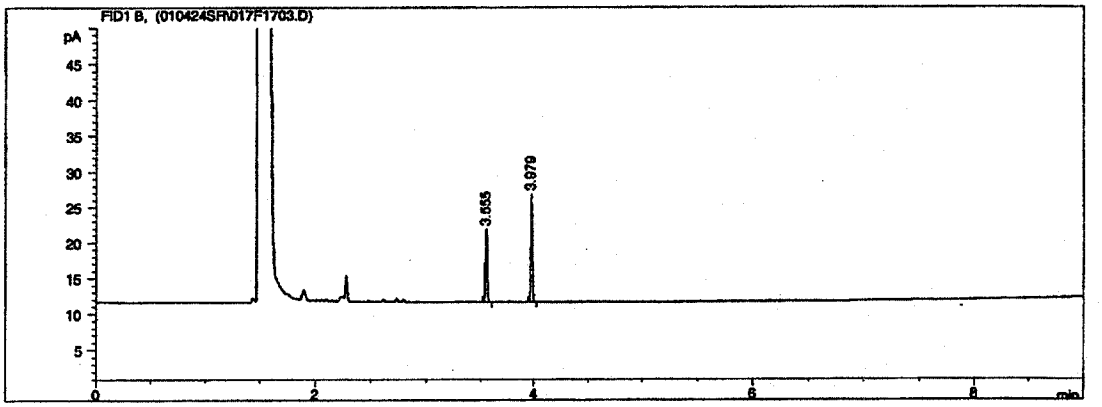

Figure 2b: Representative GC chromatogram of 25 mg/mL H-24691 dosing solution diluted to a nominal concentration of 0.03 mg/mL. The measured concentration of the representative solution is 24.2 mg/mL

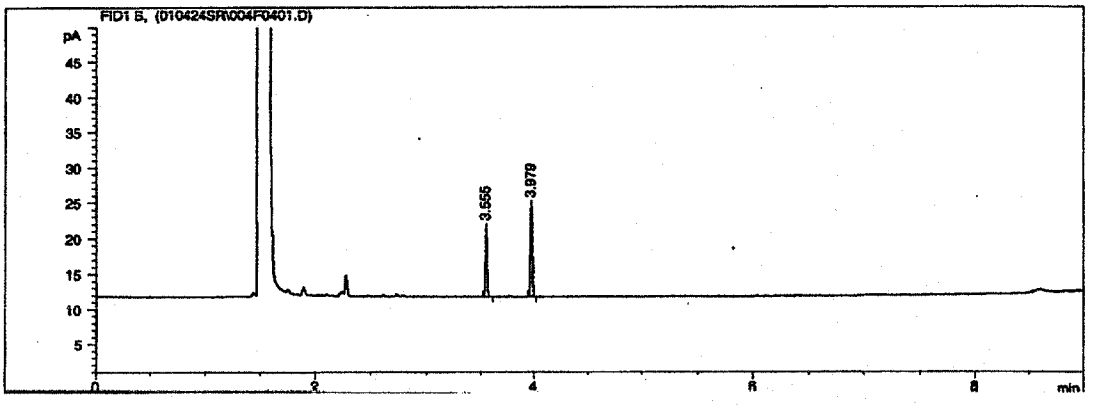

Figure 2c: Representative GC chromatogram of 0.0312 mg/mL H-24691 analytical reference solution.

TABLE 1
SUMMARY OF DOSING MIXING AND STABILITY ANALYSES

	Dosing Concentration of H-24691 (mg/mL)		
Nominal:	**1.0**	**5.0**	**25.0**
Homogeneity Samples			
Test Day 4			
Top	0.944	4.43	22.2
	(94.4)[a]	(88.6)	(88.8)
Middle	0.884	4.37	22.7
	(88.4)	(87.4)	(90.8)
Bottom	0.836[b]	4.72[b]	24.2
	(83.6)	(94.4)	(96.8)
Average Measured Conc.[c]	**0.888**	**4.51**	**23.0**
Average Percent Nominal	**(88.8)**	**(90.2)**	**(92.0)**
Standard Deviation[c]	**± 0.05**	**± 0.19**	**± 1.0**
Coefficient of Variation[c]	**6%**	**4%**	**5%**
Stability Samples			
5 Hour Room Temperature	0.854[d]	4.75	24.0
	(85.4)	(95.0)	(96.0)
Test Day 11 (7-Day Refrigerated)			
Top	0.900	4.46[e]	24.2[e]
	(90.0)[a]	(89.2)	(96.8)
Middle	0.864	4.44	23.9
	(86.4)	(88.8)	(95.6)
Bottom	0.859	---	---
	(85.9)		
Average Measured Conc.[c]	**0.874**	**4.45**	**24.1**
Average Percent Nominal	**(87.4)**	**(89.0)**	**(96.4)**
Standard Deviation[c]	**± 0.02**	**± 0.01**	**± 0.21**
Coefficient of Variation[c]	**3%**	**0.3%**	**1%**
5 Hour Room Temperature	0.904[f]	4.21[f]	21.2[f]
	(90.4)	(84.2)	(84.8)

[a] Numbers in parentheses are the respective percent of nominal values.
[b] Mean result of the original aliquot and duplicate aliquots from the original diluted sample.
[c] Statistics based on the average measured concentration (ppm) of the top, middle and bottom of each dosing level.
[d] Mean result of original aliquot, one duplicate aliquot and re-analysis of the second duplicate aliquot.
[e] Duplicate concentration verification samples reported for uniformity of mixing.
[f] Mean result of duplicate re-analysis of the original sample.

Reason For Revision No. 1

The report was revised to reflect a change in Figure 2 (page 10) of the report. The change was to correct the header and all references to "High Performance Liquid Chromatography or HPLC" to "Gas Chromatography Chromatograms or GC".

SIGNATURES

Report by: Janet C. Maslanka 13-Mar-2003

Janet C. Maslanka
Chemist

Day/Mo/Yr