AR226_3304

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DuPont Haskell Laboratory for Health
and Environmental Sciences

October 30, 2003

cc: MR [redacted]
Quality Assurance
E. Mylchreest

TO: MR FILE [redacted] POCKET H-24921

FROM: J. C. Maslanka

TEST SUBSTANCE STABILITY OF H-24921

Medical Research Project Number:	[redacted]
Haskell Sample Number:	24921
Haskell Number (Analytical Reference):	24921
Analytical Test Code:	[redacted]
Analytical Report Number:	DuPont-13707
Notebook References:	[redacted]

Attached is the analytical report to satisfy protocol requirements for studies with H-24921 but may also be used for other purposes.

TEST SUBSTANCE STABILITY OF H-24921

Medical Research Project Number: [redacted]
Haskell Sample Number: 24921
Analytical Report Number: DuPont-13707

A sample of H-24921 was received July 11, 2003, and analyzed on July 14, 2003. The percentage of active ingredient (a.i.) was measured to be [redacted] with a range of [redacted] to [redacted] of nominal for replicate analyses (n=3). The sponsor reported a purity of [redacted] when the sample was submitted.

ACKNOWLEGMENTS

Sample preparation by S. A. Riley (Chemistry Technician) and sample analysis by T. Ryan (Staff Chemist).

SIGNATURES

Report by: Janet C. Maslanka — 30-Oct-2003
Janet C. Maslanka, Senior Staff Chemist — Date

Date Issued: 30-Oct-2003

METHODS

Analysis of H-24921 was by high-performance liquid chromatography/tandem mass spectrometry (LC-MS/MS). Negative-ion electrospray is used to generate negatively charged ions of [redacted] he ions are selected with the first MS quadrupole, collisionally dissociated using argon, and a fragment ion is monitored. [redacted] s used as an internal standard.

SAMPLE PREPARATION & ANALYSIS

Aliquots (0.0300, 0.0301, and 0.0322 grams) of H-24921 were dissolved in Nanopure® water (100 mL) to the nominal concentrations of 300, 301, and 322 ppm. These were further diluted to 0.00315, 0.00316, and 0.00338 ppm with the appropriate amount of internal standard added and analyzed according to the following method.

CHROMATOGRAPHIC CONDITIONS

LC parameters

Instrument:	Hewlett Packard Model 1100 HPLC
Column:	Zorbax® RX-C8, 2.1 mm x 150 mm, 5μm
Flow Rate:	0.4 mL/min
Injection Volume:	50 μl
Column Temperature:	30°C
Column Switch:	4.0 minutes
Mobile Phase:	0.15% Acetic Acid/Acetonitrile

Gradient:

Time (min.)	% Acetonitrile
0.0	5
0.9	5
1.0	80
6.0	80
6.1	5
7.0	5

MS parameters

Instrument:	Micromass Quattro Micro Tandem MS
Ionization mode:	Electrospray (ESI), negative ion
Capillary voltage:	-2.7 kV
Cone Voltage:	15 V
Source Temperature:	120°
Desolvation Temperature:	350°
Scan function:	[redacted] 413 m/z (parent) to 369 m/z (daughter) [redacted] 415 m/z (parent) to 369 m/z (daughter)

Retention Time of [redacted] and [redacted] approximately 5.2 min (50 μL injections)

CALIBRATION & QUANTITATION

A stock solution of the H-24921 (separate sample used as analytical reference) was made in Nanopure® water. Appropriate aliquots of the stock were diluted with Nanopure® water to make calibration standards that bracketed the target concentration of the diluted sample solutions. Before these aliquots were brought to volume, an appropriate amount of internal standard [redacted] was added.

Analysis of H-24921 was by high-performance liquid chromatography/tandem mass spectrometry (LC-MS/MS). Negative-ion electrospray is used to generate negatively charged ions of [redacted] the ions are selected with the first MS quadrupole, collisionally dissociated using argon, and a fragment ion is monitored. [redacted] is used as an internal standard. Triplicate injection of the sample solutions and calibration standards solutions were made and peak areas were calculated electronically.

The calibration curve was generated by regression analysis using the peak area ratio from the H-24921 and the internal standard. Data for test solutions were compared to the calibration curves to evaluate the concentrations of the H-24921.

RESULTS

H-24921 eluted from the HPLC column as a resolved peak with a retention time of approximately 5.2 minutes for the negative ion. The sponsor reported a purity of [redacted] when the sample was submitted.

Table 1. The percent of a.i. in the H-24921 sample analyzed July 14, 2003.

Aliquot	ppm H-24921		Percent
	Targeted	Measured	Nominal
1	0.00315	0.00291	92.4
2	0.00316	0.00287	90.8
3	0.00338	0.00313	92.6
Average Percent Nominal			**91.9**
Standard Deviation			**± 1.0**
Coefficient of Variation			**1%**