FINAL REPORT

Study Title:	Analytical Validation and Stability Study of Extract, Light Paraffinic Distillate Solvent in Acetone Formulations
Study Number:	WIL-402026
Study Director:	[redacted]
Data Requirements:	Not Applicable
Study Initiation Date:	21 March 2011
Study Completion Date:	9 September 2011
Performing Analytical Laboratory:	WIL Research Laboratories, LLC 1407 George Road Ashland, OH 44805-8946
Sponsor:	American Petroleum Institute 1220 L Street, NW Washington, DC 20005

COMPLIANCE STATEMENT

This study, designated WIL-402026, was conducted in compliance with the United States EPA/TSCA GLP Standards (40 CFR Part 792), 18 September 1989; the OECD Principles of GLP [C(97) 186/Final], 26 November 1997; the WIL Research SOPs; and the protocol and protocol amendments as approved by the Sponsor with the following exception. A Certificate of Analysis was not provided by the Sponsor. It is the judgment of the Study Director that the lack of purity and expiration date had no adverse impact on the integrity of this study.

[redacted]

Manager/Research Chemist,
Analytical Chemistry
Study Director

8 Sept 2011
Date

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1. SUMMARY

A gas chromatography method using flame ionization detection for the determination of light paraffinic distillate solvent (LPDS) concentration in acetone formulations containing test substance ranging in concentration from 1.00 to 500 mg/mL was validated in this study. Test substance stability was assessed in calibration and quality control (QC) samples stored at room temperature for 24 hours and after a minimum of 48 hours. Also in this study, the assay validation was cross-validated for the determination of LPDS concentration in acetone formulations using an ethyl acetate (EtOAc) dilution to replace the hexane dilution as initially validated. Test substance stability was assessed in calibration and QC samples diluted with EtOAc and stored at room temperature for a minimum of 60 hours. In addition, test substance homogeneity and, following up to 10 days of room temperature storage, stability were assessed in formulations prepared at target concentrations of 1 and 500 mg LPDS/mL.

The LPDS assay procedure was initially validated in this study with 3 validation sessions. Quantitation was performed using calibration standards ranging in test substance concentration from 500 to 1000 µg/mL. The mean back-calculated standard concentrations had inter-session variability ranging from 1.1% to 4.2% relative standard deviation (RSD) and percent relative error (%RE) ranging from -0.64% to 0.46%, which met the protocol-specified acceptance criteria for calibration standards, *i.e.*, RSD ≤10% and %RE within ± 10% (except at the lowest level where RSD ≤15% and %RE within ± 15% were acceptable). Assay precision and accuracy were verified by the analysis of QC samples prepared at 1.00, 10.0, 100, and 500 mg LPDS/mL. The mean calculated QC concentrations had inter-session variability (precision) ranging from 0.80% to 4.2% RSD and %RE (accuracy) ranging from -1.5% to 0.0039%. The results met the protocol-specified acceptance criteria for precision and accuracy, *i.e.*, RSD ≤15% and %RE within ± 15% (except at the lowest level where RSD ≤20% and %RE within ± 20% were acceptable).

The LPDS assay procedure was cross-validated in this study with a single validation session. Quantitation was performed using calibration standards ranging in test substance concentration from 500 to 1000 µg/mL. The mean back-calculated standard concentrations had intra-session variability ranging from 0.16% to 1.2% RSD and %RE ranging from -0.44% to 0.42%, which met the previously stated acceptance criteria for calibration standards. Assay precision and accuracy were verified by the analysis of QC samples prepared at 1.00, 10.0, 100, and 500 mg LPDS/mL. The mean calculated QC concentrations had intra-session variability (precision) ranging from 0.30% to 0.82% RSD and %RE (accuracy) ranging from -0.33% to 3.0%. The results met the previously stated acceptance criteria acceptance criteria for precision and accuracy.

The test substance in calibration standards and processed QC samples prepared with hexane diluent and stored at room temperature for up to 48 hours met the WIL Research SOP acceptance criteria for stability, *i.e.*, the post-storage concentration was not <90% of the pre-storage value. The test substance in calibration standards and processed QC samples prepared with EtOAc diluent and stored at room temperature for a minimum of 60 hours met the previously stated acceptance criteria for stability.

The results of the test substance homogeneity assessment in the formulation prepared at a target concentration of 500 mg LPDS/mL met the protocol-specified acceptance criteria, *i.e.*, the RSD for the mean concentration was ≤10% at a concentration within the acceptable limits (90% to 110% of target). The results from the formulation prepared at a target concentration of 1 mg LPDS/mL met the requirement for inter-strata variability; however, the mean analyzed concentration was outside the acceptance criteria at 112% of target. Assessment of test substance resuspension homogeneity and stability in formulations prepared at target concentrations of 1 and 500 mg LPDS/mL and, following up to 10 days of room temperature storage, met the protocol-specified acceptance criteria for resuspension homogeneity, *i.e.*, the RSD for the mean concentration was ≤10%, and previously stated criteria for stability.

2. INTRODUCTION

This report provides a detailed description and validation of a gas chromatography (GC) method using flame ionization detection (FID) for the determination of light paraffinic distillate solvent (LPDS) concentration in acetone formulations containing test substance ranging in concentration from 1.00 to 500 mg/mL. Assay specificity/selectivity, calibration reproducibility, precision, accuracy, ruggedness, and test substance stability in calibration standards and processed quality control (QC) samples stored at room temperature for up to 48 hours days were assessed. The assay was cross-validated to include the use of ethyl acetate (EtOAc) as an alternate dilution solvent to the hexane used in the original validation. Test substance stability in calibration standards and processed QC samples prepared with EtOAc and stored at room temperature for up to 60 hours were assessed. In addition, formulations prepared at target concentrations of 1 and 500 mg LPDS/mL were analyzed to assess test substance homogeneity and, following up to 10 days of room temperature storage, resuspension homogeneity and stability.

The study protocol is presented in Appendix A.

A list of abbreviations potentially used in this report is presented in Section 9. (Abbreviations).

2.1. KEY STUDY DATES

Date(s)	Event(s)
5 April 2011	Experimental start/starting date (First date of analysis)
19 April 2011	Experimental termination/completion date (Last date of analysis)

2.2. WIL RESEARCH KEY STUDY PERSONNEL

[redacted]

Chemist III, Analytical Chemistry
Chemist I, Analytical Chemistry
Publishing Specialist, Reporting & Technical Support Services
Group Manager, Reporting & Technical Support Services

3. EXPERIMENTAL PROCEDURES - MATERIALS AND METHODS

3.1. TEST SUBSTANCE AND VEHICLE

3.1.1. TEST SUBSTANCE IDENTIFICATION

The test substance, LPDS, was received from EPL Archives, Inc., Sterling, VA on 10 November 2010 as follows:

Identification	Quantity Received	Physical Description
LPDS Extract Site #7, Sample #23 CAS# 64742-05-8 [WIL log no. 8470A]	4 Glass bottles	Dark brown viscous liquid

No Certificate of Analysis for the test substance was provided by the Sponsor. The purity of the test substance was specified by protocol as 100%. The test substance was stored at room temperature, protected from light and was considered stable under these conditions. A reserve sample of the test substance was collected on 15 November 2010 and stored in the WIL Research Archives.

3.1.2. VEHICLE IDENTIFICATION

The vehicle used in the preparation of the test substance formulations was acetone, identified as follows:

- Acetone, Min. 99.0% (2-propanone, CAS# 67-64-1, product code AC115, batch no. ZP3044, exp. date: 19 February 2012, received from Spectrum Chemical Mfg. Corp., New Brunswick, NJ)
- Acetone, Min. 99.0% (2-propanone, CAS# 67-64-1, product code AC115, batch no. ZE0696, exp. date: 31 March 2012, received from Spectrum Chemical Mfg. Corp., New Brunswick, NJ)

3.2. FORMULATION PREPARATION

Formulations were prepared at the test substance concentrations indicated in the following table:

Formulation Identification	Test Substance	Target Concentration (mg/mL)
Low	LPDS	1
High	LPDS	500

The appropriate amount of the test substance for each formulation was weighed in a calibrated glass container. A stir bar was added to the container and sufficient vehicle was added to each container to obtain a final volume of 75 mL. The formulations were mixed with a magnetic stirrer. The test substance formulations were stirred continuously throughout the preparation and sampling procedures.

Following formulation and sampling, each group was divided into 2 approximately 25-mL aliquots for 8-day and a minimum 10-day room temperature resuspension homogeneity and stability assessments. These aliquots were transferred to the Analytical Chemistry department for storage and resuspension

3.3. GAS CHROMATOGRAPHY

Instrument:	Agilent 6890 gas chromatograph equipped with a flame ionization detector, an Agilent 7673 autosampler and Dionex Chromeleon® data system, or equivalent
Column:	Zebron ZB-1HT Inferno column, 15 m × 0.32 mm ID, 0.25-µm film-thickness
Temperature Program:	120°C, hold for 1 minute, ramp at 40°C/minute to 400°C, hold for 1 minute
Carrier Gas:	Helium
Carrier Gas Flow Rate:	1.5 mL/minute
Injector Temperature:	300° C
Injection Volume:	1 µL split (5:1)
Detector:	Flame ionization detector at 400°C
Retention Time:	Approximately 5 minutes for LPDS
Run Time:	10 minutes

3.4. PREPARATION OF CALIBRATION STOCK SOLUTION AND STANDARDS

A calibration stock solution was prepared at a concentration of 2.00 mg LPDS/mL as follows. Approximately 20 mg of LPDS (WIL log no. 8470A, no correction for purity) was accurately weighed in a tared glass weigh funnel and transferred to a 10-mL volumetric flask with rinses of diluent. The preparation was mixed as necessary to achieve complete dissolution of the test substance. Additional diluent was added to obtain the desired concentration, and the solution was thoroughly mixed. The diluent used in the initial validation and homogeneity assessment was hexane. The diluent used in the cross-validation, homogeneity, and resuspension homogeneity/stability assessments was EtOAc.

Calibration standards at concentrations of 500, 625, 750, 875, and 1000 µg LPDS/mL were prepared by diluting aliquots of the calibration stock solution with diluent in autosampler vials. Triplicate calibration standards were prepared at each concentration for the validation sessions; at least single calibration standards at each concentration were prepared for routine analysis.

3.5. PREPARATION OF THE QUALITY CONTROL STOCK SOLUTIONS AND SAMPLES

A QC stock solution was prepared at a concentration of 20.0 mg LPDS/mL as follows. Approximately 1.0 g of LPDS (WIL log no. 8470A, no correction for purity) was accurately weighed in a tared glass weigh funnel and transferred to a 50-mL volumetric flask with rinses of diluent. The preparation was mixed as necessary to achieve complete dissolution of the test substance. Additional diluent was added to obtain the desired concentration, and the solution was thoroughly mixed. The diluent used in the initial validation and homogeneity assessment was hexane. The diluent used in the cross-validation, homogeneity, and resuspension homogeneity/stability assessments was EtOAc.

As detailed in the following table, QC samples were prepared to simulate the processing of formulations at concentrations of 1.00, 10.0, 100, and 500 mg LPDS/mL (nominal QC concentrations) by combining aliquots of the appropriate QC stock solution, vehicle (acetone), and diluent in amber autosampler vials or polypropylene tubes. The processed samples were mixed with vortex action. Portions of the samples were further diluted as necessary with diluent in autosampler vials and mixed with vortex action. The QC samples were prepared in triplicate at each concentration; a single vehicle blank sample was prepared.

QC Level	Nominal QC Concentration (mg/mL)	QC Stock Concentration (mg/mL)	QC Stock Volume (mL)	Vehicle (Acetone) Volume (mL)	Diluent Volume (mL)	Secondary Dilution	Theoretical Final Concentration (µg/mL)
Blank	0	NA	NA	0.500	0.300	NA	0
QC1	1.00	20.0	0.0250	0.500	0.275	NA	625
QC2	10.0	20.0	0.250	0.500	7.25	NA	625
QC3	100	20.0	2.50	0.500	7.00	6.67-fold	750
QC4	500	20.0	12.5	0.500	37.0	6.67-fold	750

NA = Not applicable

3.6. FORMULATION SAMPLE PROCESSING

Quadruplicate samples were collected from each formulation using a syringe and dosing cannula (initial homogeneity formulations) or class A glass volumetric pipette (repeat homogeneity formulations and resuspended aliquots) and placed in polypropylene tubes. Two samples from each quadruplicate set were processed for analysis, and the remaining 2 samples (back-up samples) were stored at room temperature and, if not needed for analysis, discarded after receipt of the Study Director's approval of the analytical results. As indicated in the following table, formulation samples were processed by adding diluent and mixing with vortex action. The diluent used in the initial homogeneity assessment was hexane. The diluent used in the repeated homogeneity and resuspension

homogeneity/stability assessments was EtOAc. Samples were further diluted as necessary with diluent in autosampler vials and mixed with vortex action.

Formulation Identification	Target Test Substance Concentration (mg/mL)	Sample Volume (mL)	Diluent Volume (mL)	Secondary Dilution	Theoretical Final Concentration (µg/mL)
Low	1	1.0	0.600	NA	625
High	500	1.0	99.0	6.67-fold	750

NA = Not applicable

3.7. CALIBRATION AND QUANTITATION

Single injections were made of each calibration standard and processed QC and formulation sample. A calibration curve was constructed for each set of analyses. The LPDS peak areas (y) and the theoretical concentrations (x) of the calibration standards were fit with least-squares regression analysis to the quadratic function:

$$y = ax^2 + b\,x + c$$

Concentrations were calculated from the results of the regression analysis using Dionex Chromeleon® software. The concentration data were transferred to a Microsoft Excel® spreadsheet, where appropriate summary statistics, *i.e.*, mean, standard deviation (SD), relative standard deviation (RSD), percent relative error (%RE), and concentration as a percent of target concentration, were calculated and presented in tabular form. The concentrations of QC and formulation samples were calculated by applying any necessary factors to correct for sample dilution or unit conversion.

3.8. WIL RESEARCH COMPUTER SYSTEMS

3.8.1. REPORTING AND ANCILLARY SYSTEMS

Program/System	Description
Archive Management System (AMS)	In-house developed application for storage, maintenance, and information retrieval for archived materials (*e.g.*, lab books, study data, wet tissues, slides, *etc.*)
InSight® Publisher	Electronic publishing system (output is Adobe Acrobat, PDF)
Master Schedule	Maintains the master schedule for the company.
Microsoft® Office 2002 and 2007; GraphPad Prism® 2008	Used in conjunction with the publishing software to generate study reports.

4. RESULTS AND DISCUSSION

Under the described chromatographic conditions, the retention time of the test substance was approximately 5 minutes. Figure 1, Figure 2, Figure 3, and Figure 4 are typical chromatograms of a calibration standard, a processed QC sample, a processed formulation sample, and a processed vehicle blank sample, respectively. The total analysis time required for each run was 10 minutes.

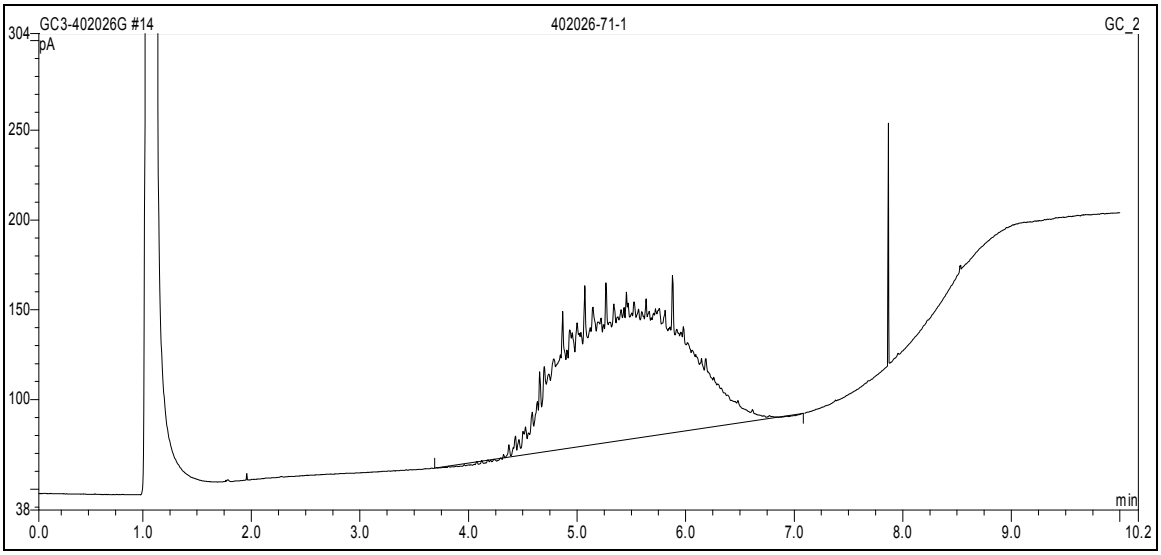

Figure 1: Representative Chromatogram of a 500 µg LPDS/mL Calibration Standard

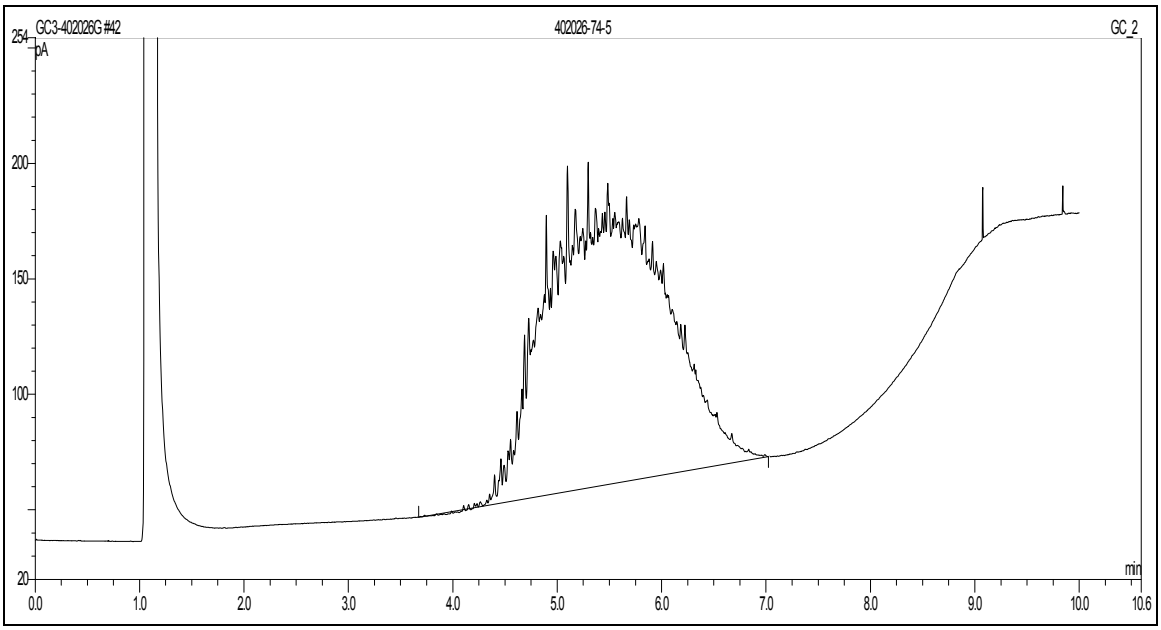

Figure 2: Representative Chromatogram of a Processed 500 mg LPDS/mL Quality Control Sample

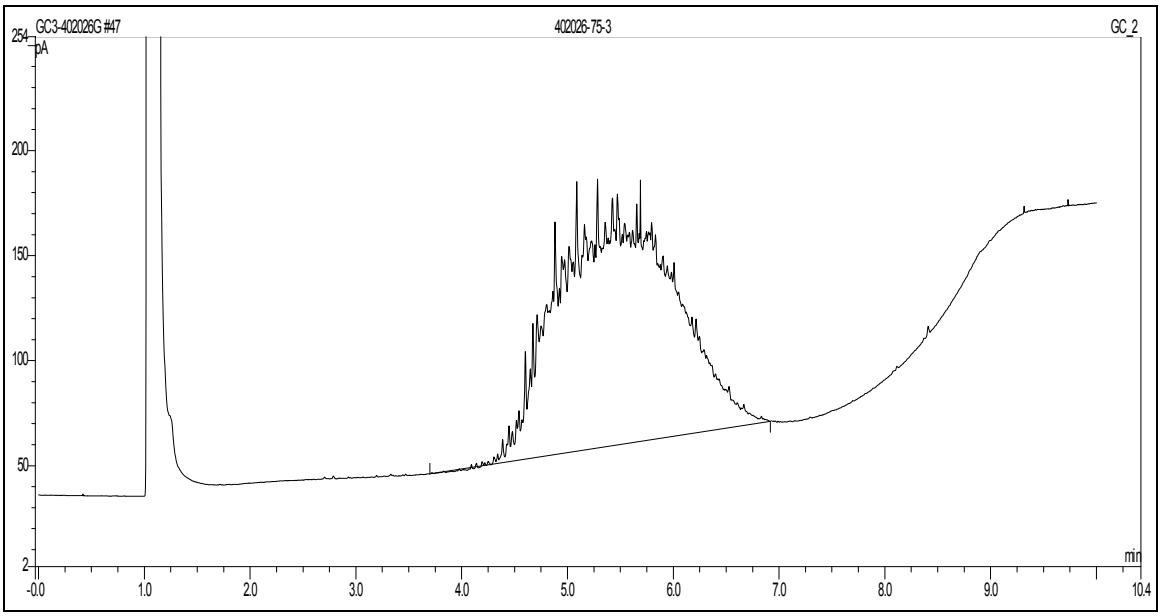

Figure 3: Representative Chromatogram of a Processed 1 mg LPDS/mL Formulation Sample

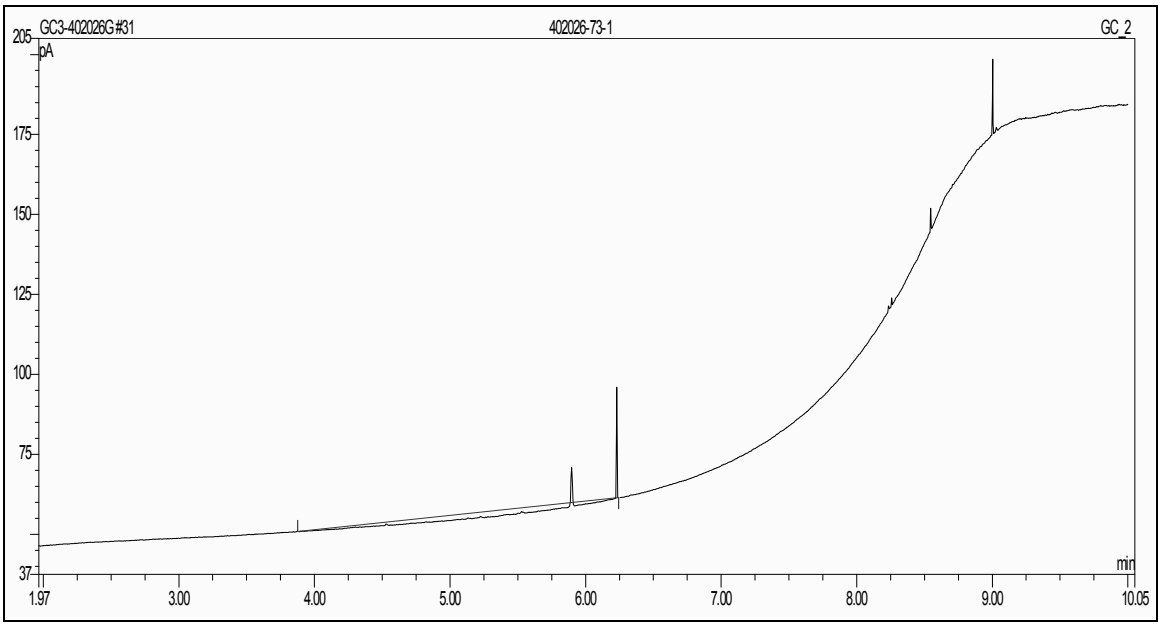

Figure 4: Chromatogram of a Processed Vehicle Blank Sample

4.1. SPECIFICITY/SELECTIVITY

As shown in Figure 4 (and in contrast to the chromatograms shown in Figure 1, Figure 2, and Figure 3), assay specificity/selectivity was confirmed when GC/FID analysis of processed vehicle samples revealed that there were no significant peaks (with signal-to-noise ratio [S/N] >10) at or near the retention time for the test substance (approximately 5 minutes).

4.2. ASSAY VALIDATION: CALIBRATION REPRODUCIBILITY

During each of 3 validation sessions, triplicate calibration standards at 5 concentrations were prepared and analyzed as described previously. Single injections were made of each calibration standard. The resulting LPDS peak area versus theoretical LPDS concentration data were fit to the quadratic function using least-squares regression analysis. The results of the regression analyses were used to back-calculate the corresponding concentrations from the peak area data. As per the study protocol, the reproducibility of the calibration curve data was considered valid when 1) the

inter-session variability, expressed as RSD, of the back-calculated concentrations at each calibration level was ≤10% RSD, except at the lowest calibration level where ≤15% was acceptable; and 2) the mean back-calculated concentrations at each calibration level were within ± 10% of the theoretical values (%RE within ± 10%), except at the lowest calibration level where %RE within ± 15% was acceptable.

The back-calculated concentrations and the associated intra- and inter-session statistics for the LPDS assay calibration standards are summarized in Table 1. The inter-session variability (RSD) of the back-calculated concentrations ranged from 1.1% to 4.2% RSD. The inter-session mean concentrations had %RE values ranging from -0.64% to 0.46%. Based on the stated criteria, the reproducibility of the calibration data was acceptable.

4.3. ASSAY CROSS-VALIDATION: CALIBRATION REPRODUCIBILITY

During the single cross-validation session, triplicate calibration standards at 5 concentrations were prepared and analyzed as described previously. Single injections were made of each calibration standard. The resulting LPDS peak area versus theoretical LPDS concentration data were fit to the quadratic function using least-squares regression analysis. The results of the regression analyses were used to back-calculate the corresponding concentrations from the peak area data. As per the study protocol, the reproducibility of the calibration curve data was considered valid when 1) the intra-session variability, expressed as RSD, of the back-calculated concentrations at each calibration level was ≤10% RSD, except at the lowest calibration level where ≤15% was acceptable and; 2) the mean back-calculated concentrations at each calibration level were within ± 10% of the theoretical values (%RE within ± 10%), except at the lowest calibration level where %RE within ± 15% was acceptable.

The back-calculated concentrations and the associated intra-session statistics for the LPDS assay calibration standards are summarized in Table 2. The intra-session variability (RSD) of the back-calculated concentrations ranged from 0.16% to 1.2% RSD.

The intra-session mean concentrations had %RE values ranging from -0.44% to 0.42%. Based on the stated criteria, the reproducibility of the calibration data was acceptable.

4.4. ASSAY VALIDATION: PRECISION AND ACCURACY

During each of 3 validation sessions, triplicate QC samples at 4 concentrations were prepared and analyzed as described previously. Single injections were made of each processed QC sample. The results of the regression analyses were used to calculate the corresponding concentrations from the QC peak area data. The variability (RSD) of the calculated QC concentration data was used as a measure of assay precision, and the difference between the theoretical and calculated mean QC concentrations (%RE) was used as a measure of assay accuracy. According to the study protocol, the precision of the method was considered acceptable when the inter-session RSD of the calculated concentrations at each QC level was ≤15%, except at the lowest concentration level where ≤20% was acceptable, and the accuracy of the method was considered acceptable when the inter-session calculated mean concentration at each QC level had a %RE value within ± 15%, except at the lowest concentration level where ± 20% was acceptable.

The calculated concentrations and the associated intra- and inter-session statistics for the LPDS assay QC samples are summarized in Table 3. The inter-session variability (RSD) of the calculated concentrations of each QC sample (precision) ranged from 0.80% to 4.2% RSD. The inter-session mean concentrations of the QC samples had %RE values (accuracy) ranging from -1.5% to 0.0039%. Based on the stated criteria, the precision and accuracy of the LPDS assay were acceptable.

4.5. ASSAY CROSS-VALIDATION: PRECISION AND ACCURACY

During the single cross-validation session, triplicate QC samples at 4 concentrations were prepared and analyzed as described previously. Single injections were made of each processed QC sample. The results of the regression analysis were used to calculate the corresponding concentrations from the QC peak area data. The variability (RSD) of the calculated QC concentration data was used as a measure of assay precision, and the

difference between the theoretical and calculated mean QC concentrations (%RE) was used as a measure of assay accuracy. As per the study protocol, the precision of the method was considered acceptable when the intra-session RSD of the calculated concentrations at each QC level was ≤15%, except at the lowest concentration level where ≤20% was acceptable, and the accuracy of the method was considered acceptable when the intra-session calculated mean concentration at each QC level had a %RE value within ± 15%, except at the lowest concentration level where ± 20% was acceptable

The calculated concentrations and the associated intra-session statistics for the LPDS assay QC samples are summarized in Table 4. The intra-session variability (RSD) of the calculated concentrations of each QC sample (precision) ranged from 0.30% to 0.82% RSD. The intra-session mean concentrations of the QC samples had %RE values (accuracy) ranging from -0.33% to 3.0%. Based on the stated criteria, the precision and accuracy of the LPDS assay were acceptable.

4.6. ASSAY RUGGEDNESS

Assay ruggedness, as required by WIL Research SOP, was successfully demonstrated for this method because at least 2 of the 3 validation sessions were performed by different analysts.

4.7. ASSAY ACCEPTABILITY

In addition to the experimental samples, each analytical session consisted of (but was not limited to) calibration standards at 5 concentrations and triplicate QC samples prepared at each of 4 concentrations. In this study, the formulations were prepared at target concentrations of 1 and 500 mg LPDS/mL, and the QC samples were prepared at nominal concentrations of 1.00, 10.0, 100, and 500 mg LPDS/mL. For an analytical session to be considered valid, at least two-thirds of the calculated QC concentrations with at least 1 sample at each concentration had to be 85% to 115% of the nominal QC concentration. All reported results were from analytical sessions that met the acceptance criteria.

4.8. TEST SUBSTANCE STABILITY IN CALIBRATION STANDARDS

Calibration standards prepared at 500 and 1000 µg/mL and analyzed on 5 April 2011 (prepared in hexane) or 16 April 2011 (prepared in EtOAc) were stored at room temperature up to approximately 48 or 60 hours, respectively, before being re-analyzed to assess test substance stability. The mean post-storage concentrations ranged from 97.2% and 111% of the pre-storage values (Table 5, Table 6, and Table 7), which met the protocol-specified requirement for stability, *i.e.*, the mean post-storage concentration was not <90% of the pre-storage value.

4.9. TEST SUBSTANCE STABILITY IN PROCESSED SAMPLES

QC samples prepared at nominal test substance concentrations of 1.00 and 500 mg/mL were processed and analyzed on 5 April 2011 (prepared in hexane) or 16 April 2011 (prepared in EtOAc). The processed samples were stored at room temperature up to approximately 48 or 60 hours, respectively, before being re-analyzed to assess test substance stability. The mean post-storage concentrations ranged from 96.8% to 103% of the pre-storage values (Table 5, Table 6, and Table 7), which met the previously stated protocol-specified requirement for stability.

4.10. TEST SUBSTANCE HOMOGENEITY AND RESUSPENSION HOMOGENEITY ASSESSMENT OF FORMULATIONS

Duplicate samples from the top, middle, and bottom strata of the formulations prepared on 6 April 2011 at target test substance concentrations of 1 and 500 mg/mL were analyzed to assess test substance homogeneity. The formulations that remained after sampling were divided into aliquots as would be used for daily dispensation. The chromatograms from the formulation samples contained additional interfering peaks not present in the calibration standards and QC samples analyzed in the same session. These peaks prevented the accurate quantitation of the LPDS test substance. The interference was attributed to the hexane solvent used to perform sample dilutions. Therefore, the assay was modified and cross-validated for dilution with EtOAc.

Duplicate samples from the top, middle, and bottom strata of the formulations prepared on 8 April 2011 at target test substance concentrations of 1 and 500 mg/mL were analyzed to assess test substance homogeneity. The formulations that remained after sampling were divided into aliquots as would be used for daily dispensation. Representative aliquots were stored at room temperature for 8 and 10 days, at which times the test substance was resuspended by stirring. Duplicate samples were collected from the top and bottom strata of the aliquots and analyzed to assess resuspension homogeneity. The results of the homogeneity and resuspension homogeneity analyses are presented in Table 8, Table 9, and Table 10 with the overall statistics summarized as follows:

Homogeneity Assessment of the 8 April 2011 Formulations

	Low Group (1 mg/mL)	High Group (500 mg/mL)
Mean Concentration (mg/mL)	1.12	494
SD	0.013	5.4
RSD (%)	1.2	1.1
Mean Concentration % of Target	112	98.7

8-Day Room Temperature Resuspension Homogeneity Assessment of the 8 April 2011 Formulations

	Low Group (1 mg/mL)	High Group (500 mg/mL)
Mean Concentration (mg/mL)	1.09	497
SD	0.0028	6.9
RSD (%)	0.26	1.4
Mean Concentration % of Target	109	99.4

10-Day Room Temperature Resuspension Homogeneity Assessment of the 8 April 2011 Formulations

	Low Group (1 mg/mL)	High Group (500 mg/mL)
Mean Concentration (mg/mL)	1.08	487
SD	0.0056	8.9
RSD (%)	0.51	1.8
Mean Concentration % of Target	108	97.3

The homogeneity assessment of the 8 April 2011 high concentration, 500 mg/mL, formulation met the protocol-specified requirement, *i.e.*, the RSD for the mean concentration was ≤10% at a concentration within the acceptable limits (within 90% to 110% of target concentration). The results from the low concentration formulation prepared at a target concentration of 1 mg LPDS/mL met the requirement for inter-strata variability; however, the mean analyzed concentration was outside the acceptance criteria at 112% of target. The resuspension homogeneity assessments of the 8 April 2011 formulations met the protocol-specified requirement, *i.e.*, the RSD for the mean concentration was 10% or less.

4.11. TEST SUBSTANCE STABILITY IN FORMULATIONS

Formulations prepared on 8 April 2011 at target concentrations of 1 and 500 mg LPDS/mL were analyzed on the day of preparation. Aliquots of the formulations were stored at room temperature for 8 and 10 days, then analyzed to assess test substance stability. The results of the stability analyses are presented in Table 9 and Table 10. The mean concentrations and percent of time-zero values are summarized in the following table.

Storage Condition	Storage Duration	Mean Concentration, mg/mL (% of Time-Zero)	
		Low (1 mg/mL)	High (500 mg/mL)
Room Temperature	8 Days	1.09 (97.6)	497 (101)
Room Temperature	10 Days	1.08 (96.7)	487 (98.5)

The post-storage test substance concentrations after 8 and 10 days at room temperature ranged from 96.7% to 101% of the pre-storage values, which met the previously-stated protocol-specified requirement for stability.

5. CONCLUSIONS

A GC/FID method for the determination of LPDS concentration in acetone formulations containing test substance ranging in concentration from 1 to 500 mg/mL was validated in this study. Method specificity/selectivity, ruggedness, calibration reproducibility, precision, accuracy, and test substance stability in calibration standards and processed QC samples stored at room temperature for 24 hours and after a minimum of 48 hours were assessed and validated, satisfying the protocol-specified acceptance criteria. The GC/FID method for the determination of LPDS concentration in acetone formulations was cross-validated using an EtOAc dilution to replace the hexane dilution that was initially validated. Method specificity/selectivity, calibration reproducibility, precision, accuracy, and test substance stability in calibration standards and processed QC samples stored at room temperature for a minimum of 60 hours were assessed and validated, satisfying the protocol-specified acceptance criteria

Formulations prepared at target test substance concentrations of 1 and 500 mg LPDS/mL met the protocol-specified requirement for homogeneity, except that the 1 mg LPDS/mL formulation was 112% of target concentration, and, after up to 10 days of room temperature storage, resuspension homogeneity and stability.

6. REPORT REVIEW AND APPROVAL

Report Prepared and Approved by:

[redacted]

9 Sept. 2011
Date

Manager/Research Chemist,
Analytical Chemistry
Study Director

Report Reviewed by:

[redacted]

9 Sept 2011
Date

Assistant Director, Analytical Chemistry

7. QUALITY ASSURANCE STATEMENT

7.1. PHASES INSPECTED

Date(s) of Inspection(s)	Phase Inspected	Dates(s) Findings Reported to Study Director	Date(s) Findings Reported to Management	Auditor(s)
18-Apr-2011	Test Article Analysis	18-Apr-2011	27-May-2011	C.Winkler
25-May-2011, 26-May-2011, 27-May-2011	Study Records (A-1)	27-May-2011	28-Jun-2011	C.Heifner
04-Aug-2011	Study Records (Rx-1)	04-Aug-2011	09-Sep-2011	C.Heifner
04-Aug-2011	Analytical Chemistry Report	04-Aug-2011	09-Sep-2011	C.Heifner
10-Aug-2011	Audited Analytical Chemistry Report	10-Aug-2011	09-Sep-2011	C.Heifner
09-Sep-2011	Final Report	09-Sep-2011	09-Sep-2011	E. Crookshank

This study was inspected in accordance with United States EPA/TSCA GLP Regulations (40 CFR Part 792), the OECD Principles of GLP, the WIL Research SOPs, and the protocol and protocol amendments as approved by the Sponsor. No Certificate of Analysis for the test substance was provided by the Sponsor. Quality Assurance findings, derived from the inspections during the conduct of the study and from the inspections of the raw data and draft report, are documented and have been reported to the Study Director. Review of the protocol and protocol amendments (if applicable) as well as a yearly internal facility inspection are conducted by the WIL Research Quality Assurance Department. A status report is submitted to management monthly.

This report accurately reflects the data generated during the study. The methods and procedures used in the study were those specified in the protocol, its amendments, and the WIL Research SOPs.

7.2. APPROVAL

This study was inspected according to the criteria discussed in Section 7.1.

Report Audited by:

[redacted]
Group Supervisor, Quality Assurance

9 Sep 2011
Date

[redacted]
Compliance Specialist

9 Sept 2011
Date

Report Released by:

[redacted]
Manager, Quality Assurance

09Sep2011
Date

8. DATA RETENTION

The raw data, the retention sample(s) if applicable, pertinent electronic storage media, and the original final report are retained in the WIL Research Archives in compliance with regulatory requirements.

9. ABBREVIATIONS

The following abbreviations may apply to this report:

µ	-	micro
µL	-	microliter
ACN	-	acetonitrile
btm	-	bottom
cm	-	centimeter
DI	-	deionized
DMSO	-	dimethylsulfoxide
EPA	-	Environmental Protection Agency
EtOAc	-	ethyl acetate
g	-	gram
GLP	-	Good Laboratory Practices
GMP	-	Good Manufacturing Practices
HPLC	-	high performance liquid chromatography
hr	-	hour(s)
IS	-	internal standard
kg	-	kilogram
L	-	liter
LLOQ	-	lower limit of quantitation
mg	-	milligram
mL	-	milliliter
mm	-	millimeter
msec	-	milliseconds
MS	-	mass spectrometry
NA	-	not applicable
ND	-	not detected
ng	-	nanogram
nm	-	nanometer
OECD	-	Organisation for Economic Cooperation and Development
ppm	-	parts per million
QC	-	quality control
%RE	-	percent relative error
RSD	-	relative standard deviation
SD	-	standard deviation
SOP	-	standard operating procedure
SPE	-	solid phase extraction
UV	-	ultraviolet
v	-	volume
w	-	weight
WIL Research	-	WIL Research Laboratories, LLC

TABLES 1 - 10

Table 1. Back-Calculated Concentrations of the Validation Calibration Standards

Concentration (µg/mL)	**500**	**625**	**750**	**875**	**1000**
Set 1 (5Apr2011)	499	631	754	869	950
	498	632	757	865	961
	494	623	756	860	1098
Mean	497	629	756	865	1003
SD	2.3	5.1	1.5	4.7	82
%RSD	0.46	0.82	0.19	0.54	8.2
%RE	-0.60	0.56	0.75	-1.2	0.27
Set 2 (6Apr2011) *Ruggedness*	506	619	734	902	998
	504	614	750	884	998
	506	617	742	900	977
Mean	505	617	742	895	991
SD	1.1	2.3	7.6	9.6	12
%RSD	0.21	0.37	1.0	1.1	1.2
%RE	1.0	-1.3	-1.1	2.3	-0.91
Set 3 (6Apr2011)	495	629	753	882	995
	515	616	762	873	1009
	499	609	747	876	991
Mean	503	618	754	877	998
SD	10	10	8.0	4.4	9.8
%RSD	2.0	1.7	1.1	0.50	0.99
%RE	0.60	-1.2	0.55	0.22	-0.17
Interset Statistics					
n	9	9	9	9	9
Mean	502	621	751	879	997
SD	6.4	8.1	8.5	14	42
%RSD	1.3	1.3	1.1	1.6	4.2
%RE	0.34	-0.64	0.080	0.46	-0.27

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Table 2. Back-Calculated Concentrations of the Cross-Validation Calibration Standards

Concentration (µg/mL)	**500**	**625**	**750**	**875**	**1000**
Cross-Validation (8Apr2011)	504	624	746	877	1006
	500	624	742	879	997
	501	619	760	880	991
Intraset Statistics					
n	3	3	3	3	3
Mean	502	622	749	879	998
SD	2.0	3.1	9.3	1.4	7.5
%RSD	0.39	0.50	1.2	0.16	0.75
%RE	0.31	-0.44	-0.10	0.42	-0.17

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Table 3. Calculated Concentrations of the Validation Quality Control Samples

Concentration (mg/mL)	**1.00**	**10.0**	**100**	**500**
Set 1 (5Apr2011)	0.937	10.1	98.1	496
	0.954	9.87	98.3	509
	0.993	10.0	99.4	517
Mean	0.961	9.99	98.6	508
SD	0.029	0.10	0.70	11
%RSD	3.0	1.0	0.71	2.1
%RE	-3.9	-0.13	-1.4	1.5
Set 2 (6Apr2011) *Ruggedness*	1.01	10.0	98.4	500
	1.00	10.0	96.9	484
	0.991	10.1	98.7	488
Mean	1.00	10.1	98.0	491
SD	0.010	0.054	0.95	8.5
%RSD	1.0	0.54	0.97	1.7
%RE	0.19	0.70	-2.0	-1.8
Set 3 (6Apr2011)	0.924	10.0	99.1	491
	1.04	9.91	98.1	487
	1.04	9.91	99.4	490
Mean	1.00	9.94	98.9	489
SD	0.066	0.056	0.69	2.3
%RSD	6.6	0.56	0.70	0.48
%RE	-0.0064	-0.56	-1.1	-2.2
Interset Statistics				
n	9	9	9	9
Mean	0.988	10.0	98.5	496
SD	0.041	0.084	0.79	11
%RSD	4.2	0.84	0.80	2.3
%RE	-1.2	0.0039	-1.5	-0.83

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Table 4. Calculated Concentrations of the Cross-Validation Quality Control Samples

Concentration (mg/mL)	**1.00**	**10.0**	**100**	**500**
Cross-Validation (8Apr2011)	1.02	10.3	101	496
	1.04	10.3	102	499
	1.03	10.1	101	500
Intraset Statistics				
n	3	3	3	3
Mean	1.03	10.2	101	498
SD	0.0072	0.084	0.300	2.2
%RSD	0.69	0.82	0.30	0.43
%RE	3.0	2.4	1.5	-0.33

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Table 5. Minimum 24-Hours Room Temperature Stability Analysis of the 5 April 2011 Calibration Standards and Processed Quality Control Samples

Date Analyzed	Theo. Conc (µg/mL)	Ref # (402026 -)	Run #	Conc (µg/mL)	Percent of Time Zero (%)	Overall Percent of Time Zero (%)
Calibration Standards						
05Apr2011	500	8 - 1	8	499	N/A	97.2
07Apr2011		8 - 1	122	485	97.1	
05Apr2011	500	8 - 2	9	498	N/A	
07Apr2011		8 - 2	123	484	97.2	
05Apr2011	1000	8 - 13	20	950	N/A	111
07Apr2011		8 - 13	124	1076	113	
05Apr2011	1000	8 - 14	21	961	N/A	
07Apr2011		8 - 14	125	1051	109	

Date Analyzed	Theo. Conc (mg/mL)	Ref # (402026 -)	Run #	Conc (mg/mL)	Percent of Time Zero (%)	Overall Percent of Time Zero (%)
QC Samples						
05Apr2011	1.00	10 - 2	25	0.937	N/A	96.8
07Apr2011		10 - 2	127	0.894	95.4	
06Apr2011	1.00	10 - 3	26	0.954	N/A	
07Apr2011		10 - 3	128	0.937	98.2	
06Apr2011	500	11 - 4	34	496	N/A	103
08Apr2011		11 - 4	129	518	104	
06Apr2011	500	11 - 5	35	509	N/A	
08Apr2011		11 - 5	130	516	101	

N/A = Not applicable

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Table 6. Minimum 48-Hours Room Temperature Stability Analysis of the
5 April 2011 Calibration Standards and Processed Quality Control Samples

Date Analyzed	Theo. Conc (µg/mL)	Ref # (402026 -)	Run #	Conc (µg/mL)	Percent of Time Zero (%)	Overall Percent of Time Zero (%)
Calibration Standards						
05Apr2011	500	8 - 1	8	499	N/A	99.2
07Apr2011		8 - 1	178	498	99.8	
05Apr2011	500	8 - 2	9	498	N/A	
07Apr2011		8 - 2	179	491	98.6	
05Apr2011	1000	8 - 13	20	950	N/A	111
07Apr2011		8 - 13	180	1061	112	
05Apr2011	1000	8 - 14	21	961	N/A	
07Apr2011		8 - 14	181	1062	110	

Date Analyzed	Theo. Conc (mg/mL)	Ref # (402026 -)	Run #	Conc (mg/mL)	Percent of Time Zero (%)	Overall Percent of Time Zero (%)
QC Samples						
05Apr2011	1.00	10 - 2	25	0.937	N/A	98.5
07Apr2011		10 - 2	182	0.895	95.5	
06Apr2011	1.00	10 - 3	26	0.954	N/A	
07Apr2011		10 - 3	183	0.968	101	
06Apr2011	500	11 - 4	34	496	N/A	102
07Apr2011		11 - 4	184	508	103	
06Apr2011	500	11 - 5	35	509	N/A	
07Apr2011		11 - 5	185	512	101	

N/A = Not applicable

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Table 7. Minimum 60-Hours Room Temperature Stability Analysis of the
16 April 2011 Calibration Standards and Processed Quality Control Samples in Ethyl Acetate

Date Analyzed	Theo. Conc (µg/mL)	Ref # (402026 -)	Run #	Conc (µg/mL)	Percent of Time Zero (%)	Overall Percent of Time Zero (%)
Calibration Standards						
16Apr2011	500	54 - 1	241	495	N/A	107
19Apr2011		54 - 1	334	540	109	
16Apr2011	500	54 - 2	242	500	N/A	
19Apr2011		54 - 2	335	526	105	
16Apr2011	500	54 - 3	243	502	N/A	
19Apr2011		54 - 3	336	529	105	
16Apr2011	1000	54 - 13	253	1006	N/A	98.3
19Apr2011		54 - 13	337	978	97.2	
16Apr2011	1000	54 - 14	254	998	N/A	
19Apr2011		54 - 14	338	990	99.2	
16Apr2011	1000	54 - 15	255	998	N/A	
19Apr2011		54 - 15	339	983	98.5	

Date Analyzed	Theo. Conc (mg/mL)	Ref # (402026 -)	Run #	Conc (mg/mL)	Percent of Time Zero (%)	Overall Percent of Time Zero (%)
QC Samples						
16Apr2011	1.00	56 - 2	260	1.08	N/A	100
19Apr2011		56 - 2	341	1.09	101	
16Apr2011	1.00	56 - 3	261	1.08	N/A	
19Apr2011		56 - 3	342	1.07	99.2	
16Apr2011	1.00	56 - 4	262	1.09	N/A	
19Apr2011		56 - 4	343	1.07	98.7	
16Apr2011	500	57 - 4	269	499	N/A	101
19Apr2011		57 - 4	344	503	101	
16Apr2011	500	57 - 5	270	498	N/A	
19Apr2011		57 - 5	345	517	104	
16Apr2011	500	57 - 6	271	503	N/A	
19Apr2011		57 - 6	346	495	98.3	

N/A = Not applicable

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Table 8. Homogeneity Assessment of the 8 April 2011 Formulations
(Analyzed 8 April 2011)

Group	Strata	Dose Conc (mg/mL)	Ref # (402026-)	Run #	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)
Low	Top	1	37 - 1	223	1.11	111	1.12	0.013	1.2	112
			37 - 2	224	1.11	111				
	Mid	1	37 - 3	225	1.13	113				
			37 - 4	226	1.13	113				
	Btm	1	37 - 5	227	1.11	111				
			37 - 6	228	1.14	114				
High	Top	500	38 - 1	229	488	97.6	494	5.4	1.1	98.7
			38 - 2	230	497	99.4				
	Mid	500	38 - 3	231	495	99.0				
			38 - 4	232	491	98.1				
	Btm	500	38 - 5	233	489	97.9				
			38 - 6	234	502	100				

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Table 9. 8-day Room Temperature Resuspension Homogeneity and Stability Assessment of the 8 April 2011 Formulations
(Analyzed 16 April 2011)

Group	Strata	Dose Conc (mg/mL)	Ref # (402026-)	Run #	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)	Percent of Time Zero (%)
Low	Top	1	58 - 1	273	1.09	109	1.09	0.0028	0.26	109	97.6
			58 - 2	274	1.10	110					
	Btm	1	58 - 3	275	1.09	109					
			58 - 4	276	1.10	110					
High	Top	500	59 - 1	277	492	98.4	497	6.9	1.4	99.4	101
			59 - 2	278	490	98.1					
	Btm	500	59 - 3	279	504	101					
			59 - 4	280	501	100					

Theoretical Conc (mg/mL)	Group	Time Zero Conc (mg/mL)
1	Low	1.12
500	High	494

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Table 10. 10-day Room Temperature Resuspension Homogeneity and Stability Assessment of the 8 April 2011 Formulations
(Analyzed 18 April 2011)

Group	Strata	Dose Conc (mg/mL)	Ref # (402026-)	Run #	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)	Percent of Time Zero (%)
Low	Top	1	75 - 1	325	1.08	108	1.08	0.0056	0.51	108	96.7
			75 - 2	326	1.09	109					
	Btm	1	75 - 3	327	1.08	108					
			75 - 4	328	1.09	109					
High	Top	500	76 - 1	329	499	99.8	487	8.9	1.8	97.3	98.5
			76 - 2	330	487	97.3					
	Btm	500	76 - 3	331	479	95.9					
			76 - 4	332	481	96.2					

Theoretical Conc (mg/mL)	Group	Time Zero Conc (mg/mL)
1	Low	1.12
500	High	494

402026 results.xls 7RH10d(rt)
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APPENDIX A

Study Protocol

Study Number: WIL-402026

PROTOCOL AMENDMENT 2

Sponsor: American Petroleum Institute

Title of Study:

Analytical Validation and Stability Study of Extract, Light Paraffinic Distillate Solvent in Acetone Formulations

Protocol Modifications:

1) **1 OBJECTIVE:**

The first paragraph in this section is modified as follows:

To develop and validate a method for the determination of aromatic distillate in acetone formulations using gas chromatography (GC) with flame ionization or mass spectrometric detection. Acetone formulations prepared at test substance concentrations of 1 and 500 mg/mL will be assessed for test substance homogeneity and, following 8 and a minimum of 10 days of room temperature storage, resuspension homogeneity and stability.

2) **8 RECORDS TO BE MAINTAINED:**

This section is modified as follows:

All original raw data records, as defined by WIL SOPs and the applicable GLPs, will be stored in the Archives at WIL Research Laboratories, LLC. Records to be retained will include, but are not limited to the following:

- Protocol and protocol amendments
- The original chromatograms, spectra and other instrument generated data
- Calculations of concentration levels and appropriate test parameters

3) **9 WORK PRODUCT:**

The first paragraph in this section is modified as follows:

The Sponsor will have title to all documentation records, raw data, and other work product generated during the performance of the study. All work product, including raw paper data and pertinent electronic storage media, will be retained at no charge for a period of six months following issuance of the final report in the Archives at WIL Research Laboratories, LLC. Thereafter, WIL Research Laboratories, LLC will charge a monthly archiving fee for retention of all work product. All work product will be stored in compliance with regulatory requirements.

Reasons for Protocol Modification:

1) Revision of this section of the protocol was inadvertently missed in amendment 1. The changes are consistent with the revisions to section 6.3.5 made by amendment 1.

2) The list of study personnel is no longer maintained in the study records.

3) Data is no longer stored on magnetic media.

Approval:

Sponsor's approval was obtained via e-mail on 29 Aug. 2011.

WIL Research Laboratories, LLC

[redacted] 29 Aug. 2011
Study Director Date

[redacted] 29 Aug 2011
Assistant Director, Analytical Chemistry Date

American Petroleum Institute

[redacted] 29 Aug 2011
Sponsor Representative Date

Study Number: WIL-402026

PROTOCOL AMENDMENT 1

Sponsor: American Petroleum Institute

Title of Study:

Analytical Validation and Stability Study of Extract, Light Paraffinic Distillate Solvent in Acetone Formulations

Protocol Modifications:

1) **6.3.5 Homogeneity, Resuspension Homogeneity, and Stability of Acetone Formulations:**

   The first two paragraphs in this section are modified as follows:

   Test substance homogeneity, resuspension homogeneity, and stability in acetone formulations prepared at test substance concentrations of 1 and 500 mg/mL will be assessed immediately after preparation and after at least 8 and a minimum of 10 days of room temperature storage. The formulations will be prepared according to instructions reviewed and authorized by the Study Director. The carrier and dose formulation preparations will be stirred during sample collection.

   For the homogeneity assessment, samples (in at least duplicate) will be collected from the top, middle, and bottom strata of the formulations on the day of preparation and analyzed to assess test substance homogeneity in the formulations. Additional samples may be collected on the day of preparation from the middle stratum and stored appropriately for the assessment of stability. Following sample collection the formulations will be divided into aliquots representative of those used for daily dispensation and stored at room temperature for 8 days and a minimum of 10 days. After the intended storage, aliquots of the formulations will be resuspended by stirring for a minimum of 30 minutes and duplicate samples from the top and bottom strata of the formulations will be collected and analyzed to assess resuspension homogeneity and, if not assessed as described above, stability.

Reasons for Protocol Modification:

1) The second resuspension homogeneity and stability assessment will be performed after a minimum of 10 days room temperature storage rather than after exactly 15 days. This is in recognition of concerns of long-term stability with the volatile acetone vehicle and so that there is flexibility in scheduling the analysis and Quality Assurance audit.

Approval:

Sponsor's approval was obtained via e-mail 12 April 2011.

WIL Research Laboratories, LLC

[redacted] 13 Apr. 2011
Study Director Date

[redacted] 13 Apr 2011
Assistant Director, Analytical Chemistry Date

American Petroleum Institute

[redacted] 13-Apr-2011
Sponsor Representative Date

PROTOCOL

ANALYTICAL VALIDATION AND STABILTIY STUDY OF EXTRACT, LIGHT PARAFFINIC DISTILLATE SOLVENT IN ACETONE FORMULATIONS

Submitted To:

American Petroleum Institute
1220 L Street, NW
Washington, DC 20005

WIL Research Laboratories, LLC
1407 George Road
Ashland, OH 44805-8946

1 OBJECTIVE:

To develop and validate a method for the determination of aromatic distillate in acetone formulations using gas chromatography (GC) with flame ionization or mass spectrometric detection. Acetone formulations prepared at test substance concentrations of 1 and 500 mg/mL will be assessed for test substance homogeneity and, following 8 and 15 days of room temperature storage, resuspension homogeneity and stability.

This study will be conducted in compliance with the U.S. EPA/TSCA, 40 CFR Part 792, and the OECD, [C(97)186/Final], Good Laboratory Practice Standards. The study will also be conducted in accordance with the protocol and WIL Research Standard Operating Procedures.

2 PERSONNEL INVOLVED IN THE STUDY:

2.1 Sponsor Representative:

[redacted]
American Petroleum Institute
1220 L Street, NW
Washington, DC 20005
Tel: (202) 682-8344
Email [redacted]

2.2 WIL Study Director:

[redacted]
Manager/Research Chemist, Analytical Chemistry
Tel: (419) 289-8700
Fax: (419) 289-3650
E-mail: [redacted]

2.3 WIL Departmental Responsibilities:

[redacted]
Research Chemist, Analytical Chemistry
Emergency Contact
Tel: (419) 289-8700
Fax: (419) 289-3650
E-mail: [redacted]

[redacted]
President and Chief Operating Officer

[redacted]
Vice President, Analytical, Metabolism, and *In Vitro* Toxicology Services

[redacted]
Manager, Quality Assurance

[redacted]
Operations Manager, Reporting and Regulatory Technical Services

3 STUDY SCHEDULE:

Proposed Experimental Starting Date:	March 2011
Proposed Experimental Completion Date:	April 2011
Proposed Audited Report Date:	Typically 6 weeks after the completion of validation activities.

4 TEST SUBSTANCE INFORMATION:

4.1 Test Substance:

4.1.1 Identification:

Extract, light paraffinic distillate solvent

Also known as aromatic distillate

4.1.2 CAS#:

64742-05-8

4.1.3 CAS definition:

A complex combination of hydrocarbons obtained as the extract from a solvent extraction process. It consists predominately of aromatic hydrocarbons having carbon numbers predominantly in the range of C15 through C30. This stream is likely to contain 5 wt. % or more of 4- to 6-membered condensed ring aromatic hydrocarbons.

4.1.4 Lot Number:

7:23

4.1.5 Expiration/Retest Date:

Retest in 5 years

4.1.6 Purity:

100%

4.1.7 Storage Conditions:

Room temperature

4.1.8 Stability:

The test substance is considered to be stable under the storage conditions provided by the Sponsor.

4.1.9 Physical Description:

To be documented by WIL Research Laboratories, LLC.

4.1.10 Reserve Samples:

Reserve samples of the test substance will be taken in accordance with WIL Standard Operating Procedures and stored in the Archives at WIL Research Laboratories, LLC indefinitely, unless otherwise specified.

4.1.11 Personnel Safety Data:

It is the responsibility of the Sponsor to notify the testing facility of any special handling requirements for the test substance. A Material Safety Data Sheet (MSDS) should accompany the test substance upon arrival at the laboratory.

4.1.12 Test Substance Disposition:

With the exception of the reserve sample for each batch of test substance, all neat test substance remaining at study completion will be returned to the Sponsor. Alternatively, the test substance can be retained for subsequent studies.

5 TEST SYSTEM:

- Acetone (as specified in Section 6.2.2) with and without test substance

6 EXPERIMENTAL DESIGN:

6.1 Overview of the Study:

Extract, light paraffinic distillate solvent is the test substance for this study and will be referred to as the analyte. The method to be validated is for the determination of the analyte concentration in acetone formulations. This study will provide the necessary data that demonstrates the analytical method as valid.

6.2 Method Details

6.2.1 Instrument

A GC equipped with a mass spectrometer and/or flame ionization detector, an autosampler, and MS workstation software, or equivalent system. Possible systems include:

- Varian 3800 GC System
- Varian 2200 Ion-Trap mass spectrometer

6.2.2 Carrier:

Acetone, Min. 99.0% (2-propanone, CAS# 67-64-1, Spectrum Chemical Mfg. Corp., product code AC115)

6.2.3 Method:

The method validation activities include two phases: (1) method evaluation and development, and (2) formal method validation.

Method evaluation of sponsor-supplied methodology usually includes (but is not limited to) the following activities: (1) the analysis of standards prepared in an appropriate solvent to establish chromatography, including retention times, resolution, sensitivity, and to check proportionality of response; (2) the analysis of the analyte prepared in the matrix to confirm the presence or absence of interferences, to evaluate potential stability limitations, and to evaluate response proportionality. Sponsor supplied methodology and other literature will be used as a starting point for method evaluation/development. Method development/evaluation will not be audited by the WIL Quality Assurance Unit.

6.3 Study Details and Criteria:

6.3.1 Specificity:

The specificity of the method will be determined by analyzing representative blank samples. The retention time window(s) corresponding to the analyte and internal standard (if applicable) will be examined for interferences and, if needed, appropriate efforts to minimize interfering peaks will be taken such as: adjustment or change of chromatographic parameters to maximize resolution of interference and analyte peaks; use of a more analyte-specific wavelength; and change in sample preparation procedure to minimize the presence of the interference in the sample to be analyzed. The success of these efforts will be determined when the method validation either passes or fails the accuracy and precision acceptance criteria for calibration and quality control samples.

6.3.2 Calibration Reproducibility:

A minimum of 3 validation sessions will be performed to validate the method for the determination of analyte concentration in the vehicle (acetone) formulations. For each validation session, at least triplicate calibration standards at a minimum of 5 different analyte concentrations will be prepared and analyzed. The concentration of the calibration standards and the regression model used for the regression analysis will be specified in the written method to be validated. The results of the regression analysis will be used to back-calculate the calibration standard concentrations. The inter-session back-calculated concentration data at each calibration level must be precise (RSD less than or equal to 10%, except at the lowest concentration level where it should not exceed 15%) and accurate (percent relative error [%RE] within ± 10% except at the lowest concentration level where it should not exceed ± 15%).

6.3.3 Accuracy and Precision:

Quality control samples will be prepared at a minimum of 3 concentrations in blank matrix – one near the lowest, one near the middle and one near the highest formulation concentration expected for future studies. The concentration of the QC samples will be specified in the written method to be validated. At least 3 replicate quality control samples at each concentration level will be analyzed with the calibration standards during each validation session. The inter-session accuracy and precision will be established based on the analyzed concentrations of the quality control samples. The inter-session analyzed concentration data

at each QC level must be precise (RSD less than or equal to 15%, except at the lowest concentration level where 20% is acceptable), and accurate (RE is within ± 15%, except at the lowest concentration level where ±20% is acceptable).

6.3.4 Stability:

The room temperature (or autosampler temperature if a cooled autosampler proves appropriate and necessary for adequate analyte stability) stability of processed samples will be evaluated over a minimum of 24 hours.

If a significant degradation (>10% reduction in the mean analyte concentration or response from the time zero samples) occurs under the tested conditions, then special precautions should be taken.

6.3.5 Homogeneity, Resuspension Homogeneity, and Stability of Acetone Formulations:

Test substance homogeneity, resuspension homogeneity, and stability in acetone formulations prepared at test substance concentrations of 1 and 500 mg/mL will be assessed immediately after preparation and after at least 8 and 15 days of room temperature storage. The formulations will be prepared according to instructions reviewed and authorized by the Study Director. The carrier and dose formulation preparations will be stirred during sample collection.

For the homogeneity assessment, samples (in at least duplicate) will be collected from the top, middle, and bottom strata of the formulations on the day of preparation and analyzed to assess test substance homogeneity in the formulations. Additional samples may be collected on the day of preparation from the middle stratum and stored appropriately for the assessment of stability. Following sample collection the formulations will be divided into aliquots representative of those used for daily dispensation and stored at room temperature for 8 days and 15 days. After the intended storage, aliquots of the formulations will be resuspended by stirring for a minimum of 30 minutes and duplicate samples from the top and bottom strata of the formulations will be collected and analyzed to assess resuspension homogeneity and, if not assessed as described above, stability.

In order for the formulations to be considered homogeneous, the RSD for the mean concentration of the analyzed samples must be less than or equal to 10% at a concentration within the acceptable limits (90% to 110% of the target concentration). In order for the formulations to be

considered homogeneous after resuspension, the RSD for the mean concentration of the analyzed samples must be less than or equal to 10%. In order for the test substance to be considered stable in the formulation, the post-storage assay concentration cannot be less than 90% of the pre-storage concentration.

7 QUALITY ASSURANCE:

The study will be audited by the WIL Quality Assurance Unit while in progress to assure compliance with GLP regulations, adherence to the protocol and to WIL SOP. The raw data and draft report will be audited by the WIL Quality Assurance Unit prior to submission to the Sponsor to assure that the final report accurately describes the conduct and the findings of the study.

This study will be included on the WIL master list of regulated studies.

8 RECORDS TO BE MAINTAINED:

All original raw data records, as defined by WIL SOPs and the applicable GLPs, will be stored in the Archives at WIL Research Laboratories, LLC. Records to be retained will include, but are not limited to the following:

- Protocol and protocol amendments
- A list of WIL study personnel involved in the conduct of the study
- The original chromatograms, spectra and other instrument generated data
- Calculations of concentration levels and appropriate test parameters

9 WORK PRODUCT:

The Sponsor will have title to all documentation records, raw data, and other work product generated during the performance of the study. All work product, including raw paper data and magnetically encoded records, will be retained at no charge for a period of six months following issuance of the final report in the Archives at WIL Research Laboratories, LLC. Thereafter, WIL Research Laboratories, LLC will charge a monthly archiving fee for retention of all work product. All work product will be stored in compliance with regulatory requirements.

Any work product, including documents, and samples, that are required by this protocol, its amendments, or other written instructions of the Sponsor, to be shipped by WIL Research Laboratories, LLC to another location will be appropriately packaged and labeled as defined by WIL's SOPs and delivered to a common carrier for shipment. WIL Research Laboratories, LLC will not be responsible for shipment following delivery to the common carrier.

10 REPORTS:

The final report will contain a summary, test substance data, methods and procedures, and an interpretation and discussion of the study results. The report will contain all information necessary to conform with current EPA and OECD specifications.

The contents of the report will be as follows:

- The study will be summarized in a formal report.
- Details of all experimental procedures and methods of calculation will be described.
- Sample preparation, chromatographic or other test conditions, calibration reproducibility, accuracy and precision will be detailed.
- Copies of chromatograms obtained in the analysis will be entered as appropriate.
- Any protocol or GLP deviations that may occur during the study will be detailed.
- A compliance statement and a Quality Assurance Unit statement will be included.

WIL Research Laboratories, LLC will provide one (1) electronic copy of an Audited Draft Report, submitted 6-8 weeks upon completion of the study prior to issuance of the final report. One (1) revision will be permitted as part of the cost of the study, from which the Sponsor's reasonable revisions and suggestions will be incorporated into the Final Report as appropriate. Additional changes or revisions may be made at extra cost. It is expected that the Sponsor will review the draft report and provide comments to WIL within a two (2) month time frame following submission. WIL will submit the Final Report within one (1) month following receipt of comments. If the Sponsor's comments/authorization to finalize the report have not been received at WIL Research Laboratories, LLC within one year following submission of the draft report, WIL Research Laboratories, LLC may elect to finalize the report following appropriate written notification to the Sponsor. Two (2) electronic copies of the Final Report (PDF) will be provided; requests for additional copies of the Final Report may result in additional charges.

11 PROTOCOL MODIFICATION:

Modification of the protocol may be accomplished during the course of this study. However, no changes will be made in the study design without the verbal or written permission of the Sponsor. In the event that the Sponsor verbally requests or approves a change in the protocol, such changes will be made by appropriate documentation in the form of a protocol amendment. All alterations of the protocol

and reasons for the modification(s) will be signed by the Study Director and the Sponsor Representative.

12 PROTOCOL APPROVAL:

Sponsor approval received via email on 17 March 2011 (1).
Date

American Petroleum Institute

[redacted]
Sponsor Representative

22-March-2011
Date

WIL Research Laboratories, LLC

[redacted]
Study Director

21 Mar. 2011
Date

[redacted]
Assistant Director, Analytical Chemistry

21 Mar 2011
Date

(1) Late entry of approval date
[redacted]
9 Sept 2011