FINAL REPORT

Study Title:	Analytical Validation and Stability Study of Ultra-Low Sulfur Diesel in Mineral Oil Formulations
Study Number:	WIL-402028
Study Director:	Eric S. Bodle, PhD
Data Requirements:	Not Applicable
Study Initiation Date:	18 March 2011
Study Completion Date:	12 January 2012
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-402028, was conducted in compliance with the United States EPA 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. A Certificate of Analysis and expiration date for the test substance was not provided by the Sponsor. Neither a Certificate of Analysis nor an expiration date for the test substance was provided by the Sponsor. However, as all results met outlined acceptance criteria, the lack of this information would not be expected to affect the quality or integrity of the data or the outcome of the study

Eric S. Bodle, PhD
Assistant Director, Analytical Chemistry
Study Director

12 JAN 2012
Date

TABLE OF CONTENTS

Page

INDEX OF FIGURES

INDEX OF TABLES

INDEX OF APPENDICES

1. SUMMARY

A gas chromatography method using mass spectrometric detection with electron impact ionization and sample extraction with 65-µm polydimethylsiloxane (PDMS)/divinylbenzene (DVB) solid phase micro-extraction (SPME) fibers for the determination of ultra-low sulfur diesel (ULSD) concentration in formulations containing mineral oil and test substance ranging in concentration from 49.4 to 502 mg/mL was validated in this study. In addition, the method was cross-validated to an alternate sample extraction with 100-µm PDMS SPME fibers. Also in this study, test substance stability was assessed in calibration standards and processed quality control (QC) samples stored at room temperature for at least 8 days. In addition, formulations prepared at target concentrations of 50 and 500 mg ULSD/mL were assessed for test substance homogeneity and, following 8 and 15 days of room temperature storage, resuspension homogeneity and stability.

The ULSD assay procedure was validated in this study with 3 validation sessions. Quantitation was performed using calibration standards ranging in test substance concentration from 10.0 to 100 mg/mL. The mean back-calculated standard concentrations had inter-session variability ranging from 5.5% to 7.1% (12% at lowest concentration) relative standard deviation (RSD) and percent relative error (%RE) ranging from -1.9% to 1.7%, 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 49.4 to 502 mg ULSD/mL. The mean calculated QC concentrations had inter-session variability (precision) ranging from 7.6% to 11% RSD and %RE (accuracy) ranging from -7.1% to -2.3%. 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 ULSD 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 10.0 to 100 mg/mL. The mean back-calculated standard concentrations had intra-session variability ranging from 1.5% to 6.5% (11% at the lowest concentration) RSD and %RE ranging from -1.2% to 4.8%, 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 49.4 to 502 mg ULSD/mL. The mean calculated QC concentrations had intra-session variability (precision) ranging from 0.17% to 4.1% RSD and %RE (accuracy) ranging from -10% to -3.5%. 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 test substance in calibration standards and processed QC samples stored at room temperature for at least 8 days met the protocol-specified acceptance criteria for stability, *i.e.*, the post-storage concentration was not <90% of the pre-storage value.

The results of the test substance homogeneity assessment in formulations prepared at target concentrations of 50 and 500 mg ULSD/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) with the following exceptions. The low group (50 mg/mL) and high group (500 mg/mL) formulations prepared on 4 May 2011 were 87.6% and 82.0% of the target concentration, respectively. Both formulations were re-prepared on 9 May 2011 and met the previously stated criteria.

Assessment of test substance resuspension homogeneity and stability of the 9 May 2011 formulations following 8 days of room temperature storage at target concentrations of 50 and 500 mg ULSD/mL met the protocol-specified acceptance criteria for resuspension homogeneity, *i.e.*, the RSD for the mean concentration was ≤10% and the previously

stated protocol-specified acceptance criteria for stability. The results of the 15-day stability assessment of the 9 May 2011 low and high group formulations failed to meet the acceptance criteria, with post-storage concentrations of 75.9% and 77.0%, respectively, of the pre-storage value.

2. INTRODUCTION

This report provides a detailed description of a gas chromatography (GC) method using mass spectrometric detection (MS) with electron impact (EI) ionization for the determination of ultra-low sulfur diesel (ULSD) concentration in formulations containing mineral oil and test substance ranging in concentration from 49.4 to 502 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 at least 8 days were assessed. In addition formulations prepared at target concentrations of 50 and 500 mg ULSD/mL were analyzed to assess test substance homogeneity and, following 8 and 15 days of room temperature storage, resuspension homogeneity and stability.

The study protocol and deviations from the protocol are 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)
28 April 2011	First date of analysis (Experimental start/starting date)
25 August 2011	Last date of analysis (Experimental termination/completion date)

2.2. WIL RESEARCH KEY STUDY PERSONNEL

J. Fabricio Beltran, BA	Associate Research Chemist, Analytical Chemistry
Robert E. Boes, MS	Associate Research Chemist, Analytical Chemistry
Stephen F. Farris, Jr., BS	Chemist III, Analytical Chemistry
Gregory A. Hawks, AS	Group Supervisor, Reporting & Technical Support Services
Melissa A. Hull, BS	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, ULSD, was received from EPL Archives, Inc., Sterling, VA on behalf of American Petroleum Institute, Washington, DC on 10 November 2010 as follows:

Identification	Quantity Received	Physical Description
ULSD Blend of 7 CAS# 68334-30-5 [WIL log no. 8472A]	5 Glass bottles	Clear, yellow liquid

The test substance was stored at room temperature, protected from light and was considered stable under this condition. A reserve sample of the test substance (approximately 0.85 g) was collected on 15 November 2010 and stored in the WIL Research Archives.

3.1.2. VEHICLE IDENTIFICATION

The vehicle used in preparation of the test substance formulations was mineral oil (USP; lot no. ZH1000, exp. date: 3 March 2012) received from Spectrum Chemicals, New Brunswick, NJ.

3.2. FORMULATION PREPARATION

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

Group	Test Substance	Concentration (mg/mL)
Low	8472A	50
High	8472A	500

The appropriate amount of the test substance for each formulation was weighed in a tared, calibrated glass container. The formulations were then brought to the calibration target with vehicle. The formulations were mixed until uniform using a magnetic stirrer. The test substance formulations were stirred continuously throughout the preparation and sampling procedures.

3.3. GAS CHROMATOGRAPHY

Column:	Zebron ZB-1HT Inferno 15 m × 0.32 mm, 0.25-µm film-thickness
Temperature Program:	Initial temperature 50°C, hold for 1.0 minute Ramp 40°C/minute to 300°C, hold for 5.0 minutes
Column Pressure:	5.0 psi
Carrier Gas:	Helium
Injector Temperature:	280°C
Sample Introduction:	Solid phase micro-extraction (SPME) 65-µm polydimethylsiloxane (PDMS)/divinylbenzene (DVB) or 100-µm PDMS
Retention Time:	Approximately 2.0 to 4.0 minutes for ULSD peak group
Run Time:	12.25 minutes

3.4. SAMPLE INCUBATION, EXTRACTION, AND DESORPTION PARAMETERS

Agitator Temperature:	60.0°C
Incubation Time:	10 minutes
Agitator Speed:	750 rpm
Agitation Cycle:	2 seconds on, 4 seconds off
Extraction Time:	2 minutes
Desorption Time:	1 minute

3.5. MASS SPECTROMETRY

Acquisition Parameters

Mode:	Selected ion monitoring
Solvent Delay:	1.0 minute
Ion Source:	EI
Transfer Line Temperature:	280°C
Ion Trap:	210°C
Manifold Temperature:	60°C

Analyte	Mass (amu)
ULSD	145.7

3.6. PREPARATION OF CALIBRATION STANDARDS

Calibration standards with a concentration range of 10.0 to 100 mg ULSD/mL were prepared by diluting aliquots of ULSD (WIL log no. 8472A, density of 837 mg ULSD/mL) with mineral oil in headspace vials. Each headspace vial had a total sample volume of 1 mL. 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.

3.7. PREPARATION OF QUALITY CONTROL SAMPLES

As detailed in the following table, QC samples were prepared to simulate the processing of formulations at concentrations of 49.4, 100, and 502 mg ULSD/mL (nominal QC concentrations) by combining aliquots of ULSD (WIL log no. 8472A, density of 837 mg ULSD/mL) and vehicle (mineral oil) in headspace vials or polypropylene tubes. The processed samples were further diluted as necessary with mineral oil and mixed with vortex action. The samples were transferred to a headspace vial for analysis. Each headspace vial had a total sample volume of 1 mL. The QC samples were prepared in triplicate at each concentration; a single vehicle blank sample was prepared.

QC Level	Nominal QC Concentration (mg/mL)	Vehicle Volume (mL)	ULSD Density (mg/mL)	ULSD Volume (mL)	Dilution	Theoretical Final Concentration (mg/mL)
Blank	0	1.00	837	0	NA	0
QC1	49.4	0.941	837	0.059	NA	49.4
QC2	100	0.880	837	0.120	2-fold	50.2
QC3	502	0.400	837	0.600	10-fold	50.2

NA = Not applicable

3.8. FORMULATION SAMPLE COLLECTION AND PROCESSING

Quadruplicate samples were collected using a syringe and dosing cannula and transferred to headspace vials or 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, protected from light and if not needed for analysis, discarded after receipt of the Study Director's approval of analytical results. As indicated in the following table, formulation samples diluting as necessary with mineral oil and mixing with vortex action. The samples were transferred to a headspace vial for analysis. Each headspace vial had a total sample volume of 1 mL.

Group	Target Test Substance Concentration (mg/mL)	Sample Volume (mL)	Dilution	Theoretical Final Concentration (mg/mL)
Low	50	1.0	NA	50.0
High	500	1.0	10-fold	50.0

NA = Not applicable

3.9. CALIBRATION AND QUANTITATION

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

$$y=ax^2 + bx + c$$

Concentrations were calculated from the results of the regression analysis using Microsoft Excel®. The concentration data were transferred to another 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.

3.10. WIL RESEARCH COMPUTER SYSTEMS

3.10.1. REPORTING AND ANCILLARY SYSTEMS

Program/System	Description
Archive Management System (AMS)	In-house developed application for storage, maintenance, and retrieval of information 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 peak group was approximately 2.0 to 4.0 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 QC blank sample, respectively. The total analysis time required for each run was 12.25 minutes.

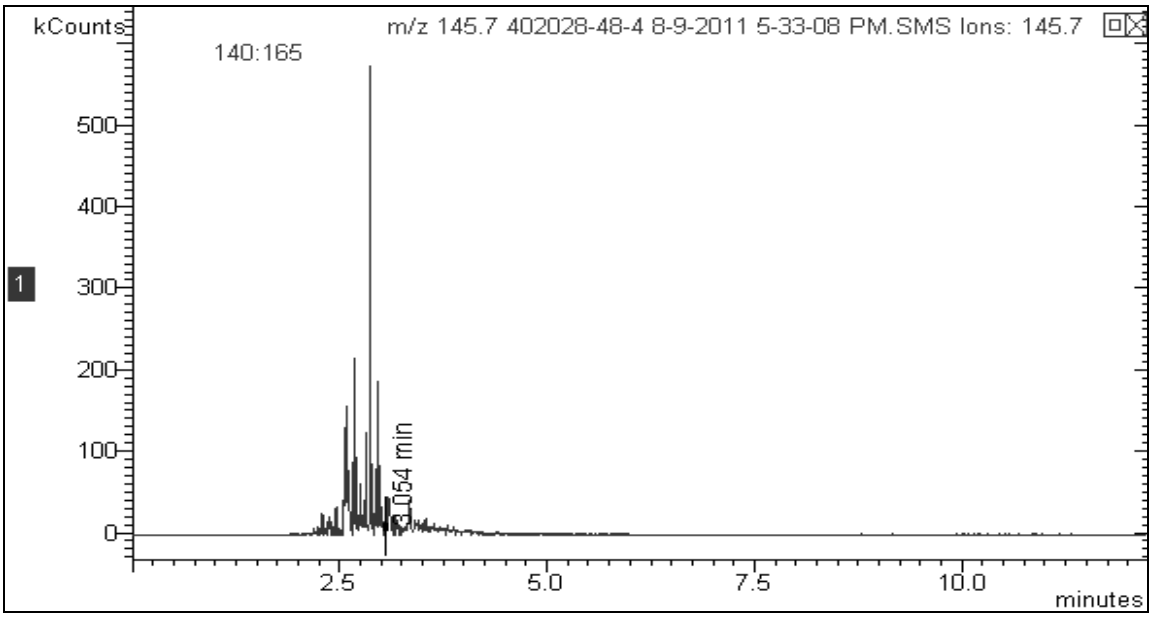

Figure 1: Representative Chromatogram of a 25.1 mg ULSD/mL Calibration Standard

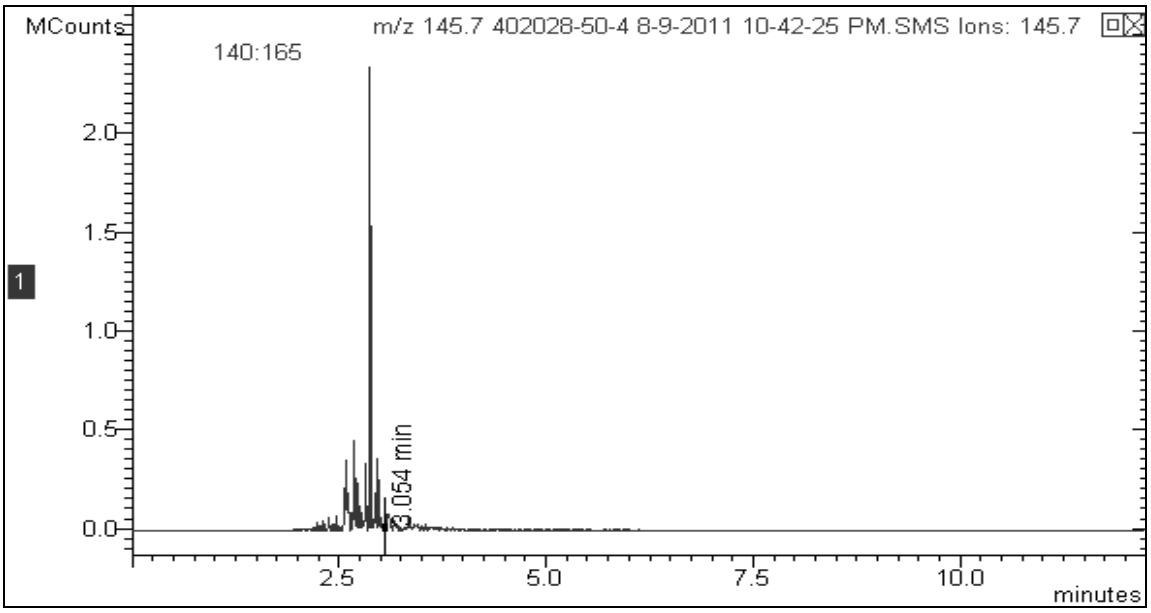

Figure 2: Representative Chromatogram of a Processed 502 mg ULSD/mL Quality Control Sample

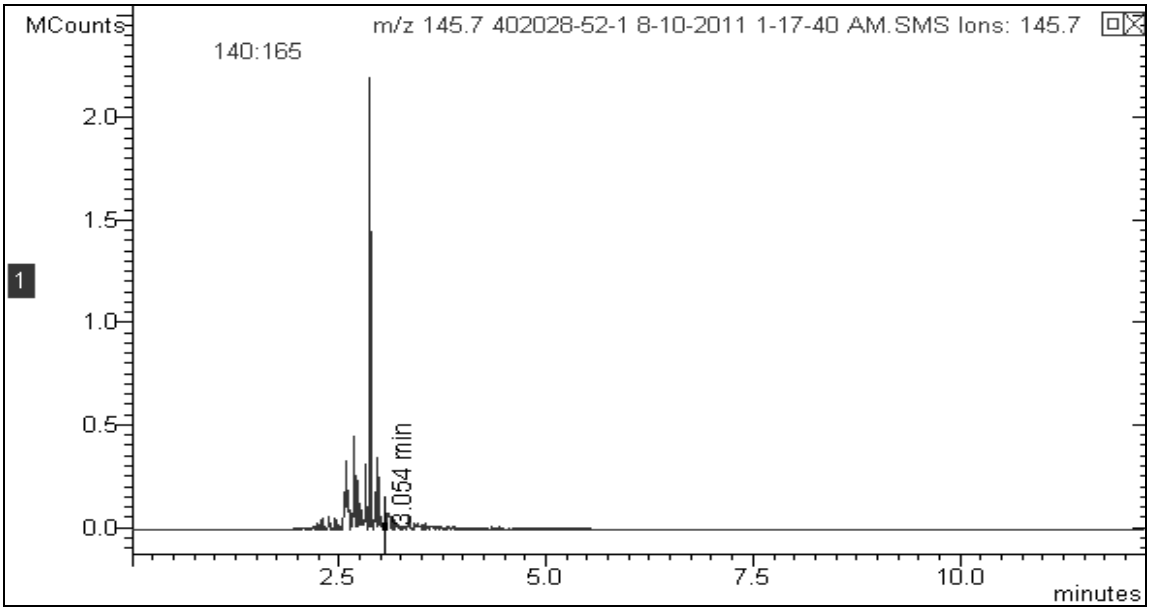

Figure 3: Representative Chromatogram of a Processed 500 mg ULSD/mL Formulation Sample

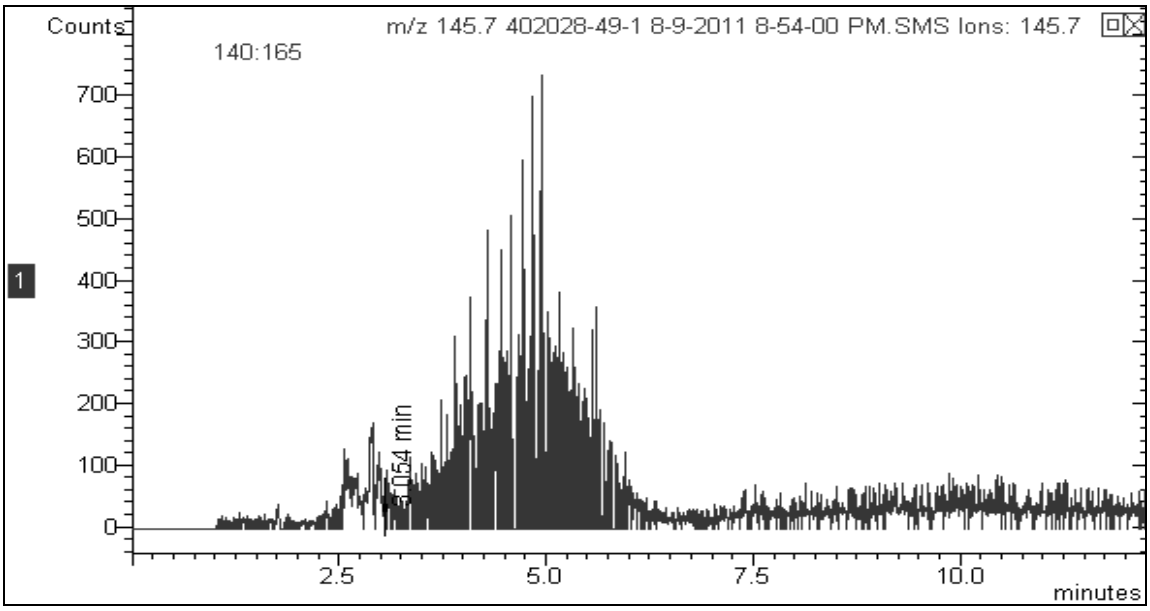

Figure 4: Chromatogram of a Processed Quality Control Blank Sample

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/MS analysis of a processed QC blank sample revealed that there were no significant peaks at or near the retention time for the test substance peak group (approximately 2.0 to 4.0 minutes).

4.2. ASSAY VALIDATION: CALIBRATION REPRODUCIBILITY

During each of the 3 method validation sessions (65-µm PDMS/DVB fibers)and the subsequent single cross-validation session (100-µm PDMS SPME fibers), triplicate calibration standards at 5 concentrations were prepared and analyzed as described previously. Single injections were made of each calibration standard. The resulting ULSD peak group areas versus theoretical ULSD 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 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%, 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 (percent relative error [%RE] within ± 10%), except at the lowest calibration level where %RE within ± 15% was acceptable. Intra-session statistics were used to evaluate the single cross-validation session.

The back-calculated concentrations and the associated inter- and/or intra-session statistics for the ULSD assay validation and cross-validation calibration standards are summarized in Table 1 and Table 2, respectively, with the inter- or intra-session variability (RSD) of the back-calculated concentrations and the %RE of the inter- or intra-session mean concentrations summarized as follows.

Validation	RSD Range of Values (%)	%RE Range of Values (%)
Full (3 sessions)	5.5 to 7.1 (12% at lowest conc.)	-1.9 to 1.7
Cross- (1 session)	1.5 to 6.5 (11% at lowest conc.)	-1.2 to 4.8

Based on the stated criteria, the reproducibility of the ULSD calibration data was acceptable.

4.3. ASSAY VALIDATION: PRECISION AND ACCURACY

During each of the 3 method validation sessions and the subsequent single cross-validation session, triplicate QC samples at 3 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 theoretical and the calculated mean QC concentrations (%RE) was used as a measure of

assay accuracy. According to the protocol, the precision of the method was considered acceptable when the inter-session RSD of the calculated concentrations at each QC level was ≤15%, 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 level where RSD ≤20% and %RE within ± 20% were acceptable). Intra-session statistics were used to evaluate the single cross-validation session.

The calculated concentrations and the associated inter- and/or intra-session statistics for the ULSD assay validation and cross-validation QC samples are summarized in Table 3 and Table 4, respectively, with the inter- or intra-session variability (RSD) of the calculated concentrations of each QC sample (precision) and the %RE values (accuracy) of the inter- or intra-session mean concentrations of the QC samples summarized as follows.

Validation	QC Range (mg/mL)	RSD Range of Values (%)	%RE Range of Values (%)
Full (3 sessions)	49.4 to 502	7.6 to 11	-7.1 to -2.3
Cross- (1 session)	49.4 to 502	0.17 to 4.1	-10 to -3.5

Based on the previously stated criteria, the precision and accuracy of the ULSD assay was acceptable

4.4. 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.

4.5. ASSAY ACCEPTABILITY

In addition to the experimental samples, each analytical session consisted of (but was not limited to) calibration standards at a minimum of 4 concentrations and triplicate QC samples prepared at each of 3 concentrations. In this study, the formulations were

prepared at target concentrations of 50 and 500 mg ULSD/mL, and the QC samples were prepared at nominal concentrations of 49.4, 100, and 502 mg ULSD/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.

4.6. TEST SUBSTANCE STABILITY IN CALIBRATION STANDARDS

Calibration standards prepared at 25.1 and 100 mg/mL and analyzed on 9 August 2011 were stored at room temperature for 8 days before being re-analyzed to assess test substance stability. The mean post-storage concentrations were 96.7% and 95.4% of the pre-storage values (Table 5), which met the protocol-specified requirement for stability, *i.e.*, the mean post-storage concentration was not <90% of the pre-storage value.

4.7. TEST SUBSTANCE STABILITY IN PROCESSED SAMPLES

QC samples prepared at nominal test substance concentrations of 1.00 and 200 mg/mL were processed and analyzed on 9 August 2011. The processed samples were stored at room temperature for 8 days before being re-analyzed to assess test substance stability. The mean post-storage concentrations were 97.1% and 97.3% of the pre-storage values (Table 5), which met the previously stated protocol-specified requirement for stability.

4.8. TEST SUBSTANCE HOMOGENEITY ASSESSMENT OF FORMULATIONS

Duplicate samples from the top, middle, and bottom strata of the formulations prepared on 4 May 2011 at target test substance concentrations of 50 and 500 mg/mL were analyzed to assess test substance homogeneity. The results of the homogeneity analysis are presented in Table 6, with the overall statistics summarized as follows:

Homogeneity Assessment of the 4 May 2011 Formulations		
	Low Group (50 mg/mL)	High Group (500 mg/mL)
Mean Concentration (mg/mL)	43.8	410
SD	3.0	19
RSD (%)	6.8	4.6
Mean Concentration % of Target	87.6	82.0

The homogeneity assessment of the 4 May 2011 formulations 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) with the following exceptions. The low group (50 mg/mL) and high group (500 mg/mL) formulations prepared on 4 May 2011 were 87.6% and 82.0% of the target concentration, respectively.

4.9. TEST SUBSTANCE HOMOGENEITY AND RESUSPENSION HOMOGENEITY ASSESSMENT OF FORMULATIONS

Duplicate samples from the top, middle, and bottom strata of the formulations prepared on 9 August 2011 at target test substance concentrations of 50 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 15 days, at which time the test substance was resuspended by stirring. Duplicate samples were collected from the top and bottom strata of the aliquots and analyzed to assess 8 and 15 day resuspension homogeneity. The results of the homogeneity and resuspension homogeneity analyses are presented in Table 7, Table 8, and Table 9, respectively, with the overall statistics summarized as follows:

Homogeneity Assessment of the 9 August 2011 Formulations		
	Low Group (50 mg/mL)	High Group (500 mg/mL)
Mean Concentration (mg/mL)	54.2	537
SD	3.3	9.4
RSD (%)	6.0	1.8
Mean Concentration % of Target	108	107

8-Day Room Temperature Resuspension Homogeneity Assessment of the 9 August 2011 Formulations		
	Low Group (50 mg/mL)	High Group (500 mg/mL)
Mean Concentration (mg/mL)	51.8	540
SD	3.6	14
RSD (%)	7.0	2.7
Mean Concentration % of Target	104	108

15-Day Room Temperature Resuspension Homogeneity Assessment of the 9 August 2011 Formulations		
	Low Group (50 mg/mL)	High Group (500 mg/mL)
Mean Concentration (mg/mL)	42.2	439
SD	3.0	21
RSD (%)	7.0	4.8
Mean Concentration % of Target	84.5	87.7

The homogeneity assessment of 9 August 2011 formulations met the previously stated protocol-specified requirement. The resuspension homogeneity assessments of the 9 August 2011 formulations met the protocol-specified requirement, *i.e.*, the RSD for the mean concentration was ≤10%.

4.10. TEST SUBSTANCE STABILITY IN FORMULATIONS

The formulations prepared and analyzed on 9 August 2011 were stored at room temperature, protected from light for 8 and 15 days before being re-analyzed to assess test substance stability. The results of the stability analysis are presented in Table 10 and Table 11. The mean concentrations and percent of time-zero values are summarized in the following table.

		Mean Concentration, mg/mL (% of Time-Zero)	
Storage Condition	Storage Duration	Low Group (50 mg/mL)	High Group (500 mg/mL)
Room Temperature	8 Days	51.8 (95.6)	521 (96.9)
	15 Days	41.1 (75.9)	413 (77.0)

The post-storage test substance concentrations following 8 days of room temperature storage ranged from 95.6% to 96.9% of the pre storage values, which met the previously stated protocol requirement for stability. The post-storage test substance concentrations following 15 days of room temperature storage ranged from 75.9% to 77.0% of the pre-storage values, which did not meet the previously stated acceptance criteria.

5. CONCLUSIONS

A GC/MS method using EI ionization and sample extraction with 65-µm PDMS/DVB SPME fibers for the determination of ULSD concentration in formulations containing mineral oil and test substance ranging in concentration from 49.4 to 502 mg/mL was validated in this study. In addition, the method was cross-validated to an alternate sample extraction with 100-µm PDMS SPME fibers. Method specificity/selectivity, ruggedness, calibration reproducibility, precision, accuracy, and test substance stability in calibration standards and processed QC samples stored at room temperature for at least 8 days were assessed and validated, satisfying WIL Research SOP and protocol acceptance criteria.

The results of the test substance homogeneity assessment in formulations prepared at target concentrations of 50 and 500 mg ULSD/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) with the following exceptions. The low group (50 mg/mL) and high group (500 mg/mL) formulations prepared on 4 May 2011 were 87.6% and 82.0% of the target concentration, respectively.

Assessment of test substance resuspension homogeneity and stability following 8 and 15 days of room temperature storage at target concentrations of 50 and 500 mg ULSD/mL met the protocol-specified acceptance criteria with the following exception. The results of the 15-day stability assessment of the 9 August 2011 low and high group formulations failed to meet the acceptance criteria.

6. REPORT REVIEW AND APPROVAL

Report Reviewed and Approved by:

Eric S. Bodle, PhD
Assistant Director, Analytical Chemistry
Study Director

12 Jan 2012
Date

Report Prepared by:

Robert E. Boes, MS
Associate Research Chemist,
Analytical Chemistry

12 Jan 2012
Date

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)
09-Aug-2011	Test Article Analysis	09-Aug-2011	26-Sep-2011	M.Stauffer
19-Sep-2011	Study Records (Rx-1, Rx-2)	19-Sep-2011	25-Oct-2011	M.Stauffer
19-Sep-2011, 20-Sep-2011	Study Records (A-1, A-2, A-3)	20-Sep-2011	25-Oct-2011	M.Stauffer
23-Sep-2011	Analytical Chemistry Report	23-Sep-2011	25-Oct-2011	M.Stauffer
17-Oct-2011	Audited Analytical Chemistry Report	17-Oct-2011	28-Nov-2011	M.Stauffer
09-Jan-2012	Final Report	09-Jan-2012	10-Jan-2012	E.Crookshank

This study was inspected in accordance with the United States EPA 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. 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:

Elizabeth B. Crookshank, BS
Group Supervisor, Quality Assurance
12 Jan 2012
Date

Mary L. Stauffer, BS
Senior Compliance Specialist
12 Jan. 12
Date

Report Released by:

Heather L. Johnson, BS, RQAP-GLP
Manager, Quality Assurance
12 Jan 2012
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
amu	-	atomic mass unit
btm	-	bottom
conc.	-	concentration
DI	-	deionized
DVB	-	divinylbenzene
EI	-	electron impact
EPA	-	Environmental Protection Agency
ESI+	-	positive electrospray ionization
g	-	gram
GLP	-	Good Laboratory Practices
hr	-	hour(s)
IS	-	internal standard
kg	-	kilogram
L	-	liter
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
PDMS	-	polydimethylsiloxane
ppm	-	parts per million
QC	-	quality control
%RE	-	percent relative error
RSD	-	relative standard deviation
SD	-	standard deviation
SOP	-	standard operating procedure
SPME	-	solid phase micro-extraction
ULSD	-	ultra-low sulfur diesel
v	-	volume
w	-	weight
WIL Research	-	WIL Research Laboratories, LLC

TABLES 1 - 11

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

Theoretical Concentration (mg/mL)	**10.0**	**25.1**	**50.2**	**75.3**	**100**
Set 1 (28 Apr 2011)	8.49	24.5	55.0	79.1	104
	10.5	23.8	48.1	73.8	101
	11.3	24.0	53.8	67.5	97.0
Mean	10.1	24.1	52.3	73.5	100
SD	1.5	0.39	3.7	5.8	3.4
%RSD	14	1.6	7.1	7.9	3.4
%RE	1.0	-4.1	4.1	-2.4	0.48
Set 2 (29 Apr 2011)	11.3	21.1	50.5	187*	89.3
	10.6	24.9	50.2	81.4	109
	11.3	25.1	47.9	74.4	99.9
Mean	11.1	23.7	49.5	77.9	99.2
SD	0.42	2.3	1.4	5.0	9.6
%RSD	3.8	9.6	2.8	6.4	9.7
%RE	11	-5.6	-1.3	3.4	-0.76
Set 3 (4-5 May 2011) *(Ruggedness)*	8.95	26.0	65.0*	72.5	104
	4.64*	24.9	54.6	76.9	96.1
	8.97	27.3	44.9	75.9	100
Mean	8.96	26.1	49.7	75.1	100
SD	0.019	1.2	6.8	2.3	3.9
%RSD	0.22	4.4	14	3.1	3.9
%RE	-10	3.9	-0.94	-0.27	0.072
Interset Statistics					
n	8	9	8	8	9
Mean	10.2	24.6	50.6	75.2	99.9
SD	1.2	1.7	3.6	4.3	5.5
%RSD	12	6.9	7.1	5.7	5.5
%RE	1.7	-1.9	0.82	-0.15	-0.069

* Standards will be excluded from summary statistics due to suspected preparation errors

402028Quad.xls I
Printed: 01/11/12 1:25 PM

Table 2. Back-Calculated Concentrations of the Cross-Validation Calibration Standards
Cross to 100 µm PDMS SPME Fibers

Theoretical Concentration (mg/mL)	**10.0**	**25.1**	**50.2**	**75.3**	**100**
Cross-Validation (17 June 2011)	9.69	23.8	50.7	77.2	92.3
	11.3	26.2	49.2	74.9	103
	13.0*	24.3	49.8	75.0	104
Intraset Statistics					
n	2	3	3	3	3
Mean	10.5	24.8	49.9	75.7	99.8
SD	1.1	1.3	0.76	1.3	6.5
%RSD	11	5.1	1.5	1.7	6.5
%RE	4.8	-1.2	-0.57	0.54	-0.23

*Calibration standard excluded from summary statistics due to suspected preparation error

Table 3. Calculated Concentrations of the Validation Quality Control Samples

Theoretical Concentration (mg/mL)	**49.4**	**100**	**502**
Set 1 (28 Apr 2011)	47.4	109	544
	49.0	102	549
	53.3	88.9	451
Mean	49.9	99.9	515
SD	3.1	10	55
%RSD	6.1	10	11
%RE	0.98	-0.099	2.6
Set 2 (29 Apr 2011)	50.0	91.3	487
	47.0	88.2	537
	53.6	103	503
Mean	50.2	94.2	509
SD	3.3	7.9	26
%RSD	6.5	8.4	5.0
%RE	1.6	-5.8	1.4
Set 3 (4-5 May 2011) *(Ruggedness)*	47.3	86.7	410
	43.5	84.3	430
	43.2	82.6	437
Mean	44.7	84.5	426
SD	2.3	2.1	14
%RSD	5.1	2.4	3.3
%RE	-9.6	-15	-15
Interset Statistics			
n	9	9	9
Mean	48.2	92.9	483
SD	3.7	9.4	53
%RSD	7.6	10	11
%RE	-2.3	-7.1	-3.7

402028Quad.xls II
Printed: 01/11/12 1:25 PM

WIL-402028
American Petroleum Institute

Table 4. Back-Calculated Concentrations of the Cross-Validation Quality Control Samples
Cross to 100 µm PDMS SPME Fibers

Theoretical Concentration (mg/mL)	**49.4**	**100**	**502**
Cross-Validation (17 June 2011)	49.5	92.9	450
	47.8	93.2	464
	45.6	93.2	442
Intraset Statistics			
n	3	3	3
Mean	47.6	93.1	452
SD	1.9	0.16	11
%RSD	4.1	0.17	2.5
%RE	-3.5	-6.9	-10

Table 5. Minimum 8-Day Room Temperature Stability Analysis of the
9 August 2011 Calibration Standards and Processed Quality Control Samples

Date Analyzed	Theo. Conc (mg/mL)	Ref # (402028 -)	Line # *	Conc (mg/mL)	Percent of Time Zero (%)	Overall Percent of Time Zero (%)
Calibration Standards						
09Aug2011	25.1	48 - 4	9	26.5	N/A	96.7
18Aug2011		48 - 4	45	24.6	92.7	
09Aug2011	25.1	48 - 5	10	22.8	N/A	
18Aug2011		48 - 5	46	23.0	101	
09Aug2011	100	48 - 13	18	101	N/A	95.4
18Aug2011		48 - 13	47	95.9	95.2	
09Aug2011	100	48 - 14	19	96.8	N/A	
18Aug2011		48 - 14	48	92.5	95.6	

Date Analyzed	Theo. Conc (mg/mL)	Ref # (402028 -)	Line # *	Conc (mg/mL)	Percent of Time Zero (%)	Overall Percent of Time Zero (%)
QC Samples						
09Aug2011	1.00	49 - 2	23	55.9	N/A	97.1
18Aug2011		49 - 2	50	54.8	98.0	
09Aug2011	1.00	49 - 3	24	53.6	N/A	
18Aug2011		49 - 3	51	51.6	96.2	
09Aug2011	200	50 - 4	29	544	N/A	97.3
18Aug2011		50 - 4	52	550	101	
09Aug2011	200	50 - 5	30	575	N/A	
18Aug2011		50 - 5	53	537	93.5	

* The line number for prestorage samples injected on 9Aug2011 shall be prefixed by sequence (402028h-) and the line number for poststorage stability samples injected on 18Aug2011 shall be prefixed by sequence (402028i-)

N/A = Not applicable

402028Quad.xls 4pss2d(rt)
Printed: 01/11/12 1:25 PM

Table 6. Homogeneity/Concentration Assessment of the 4 May 2011 Formulations
(Analyzed 4-6 May 2011)

Group/ Strata	Dose Conc. (mg/mL)	Ref # (402028 -)	Line # *(402028d1-) ^(402028e-)	Analyzed Conc. (mg/mL)	Percent of Target (%)	Mean Conc. (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)
Low/Top	50	11 - 1	34*	38.5	77.0	43.8	3.0	6.8	87.6
		11 - 2	35*	43.0	86.0				
		13 - 1	57^	43.8	87.7				
		13 - 2	58^	48.2	96.4				
Low/Mid		11 - 3	36*	44.4	88.8				
		11 - 4	37*	41.2	82.4				
		13 - 3	59^	47.2	94.4				
		13 - 4	60^	45.3	90.6				
Low/Btm		11 - 5	38*	43.9	87.9				
		11 - 6	39*	40.3	80.5				
		13 - 5	61^	47.5	95.0				
		13 - 6	62^	42.2	84.4				
High/Top	500	12 - 1	40*	428	85.5	410	19	4.6	82.0
		12 - 2	41*	410	82.1				
		21 - 1	63^	421	84.2				
		21 - 2	64^	398	79.6				
High/Mid		12 - 3	42*	413	82.7				
		12 - 4	43*	400	79.9				
		21 - 3	65^	405	81.0				
		21 - 4	66^	396	79.2				
High/Btm		12 - 5	44*	447	89.3				
		12 - 6	45*	399	79.8				
		21 - 5	67^	430	86.0				
		21 - 6	68^	376	75.2				

Page 11 and 12 samples collected/processed on 4 May 2011 and stored at room temperature and injected on 5 May 2011
Page 13 and 21 samples are back-up samples collected on 4 May 2011 and stored at room temperature until injection/processing on 6 May 2011

402028Quad.xls 1H
Printed: 11Jan2012 1:25 PM

Table 7. Homogeneity/Concentration Assessment of the 9 August 2011 Formulations
(Analyzed 9 August 2011)

Group/ Strata	Dose Conc. (mg/mL)	Ref # (402028 -)	Line # (402028h-)	Analyzed Conc. (mg/mL)	Percent of Target (%)	Mean Conc. (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)
Low/Top	50	51 - 1	33	58.6	117	54.2	3.3	6.0	108
		51 - 2	34	51.4	103				
Low/Mid		51 - 3	35	52.7	105				
		51 - 4	36	52.7	105				
Low/Btm		51 - 5	37	58.1	116				
		51 - 6	38	51.5	103				
High/Top	500	52 - 1	39	546	109	537	9.4	1.8	107
		52 - 2	40	545	109				
High/Mid		52 -3*	41	658	132				
		52 - 4	42	540	108				
High/Btm		52 - 5	43	525	105				
		52 - 6	44	529	106				

* Sample excluded from summary statistics due to presence of unknown peak in chromatogram

402028Quad.xls 2H
Printed: 11Jan2012 1:25 PM

Table 8. 8-Day Room Temperature Resuspension Homogeneity Assessment of the 9 August 2011 Formulations
(Analyzed 17-18 August 2011)

Group/ Strata	Dose Conc. (mg/mL)	Ref # (402028 -)	Line # (402028i-)	Analyzed Conc. (mg/mL)	Percent of Target (%)	Mean Conc. (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)
Low/Top	50	66 - 1	30	56.3	113	51.8	3.6	7.0	104
		66 - 2	31	52.8	106				
Low/Btm		66 - 3	32	47.8	96				
		66 - 4	33	50.2	100				
High/Top	500	67 - 1	35	540	108	540	14	2.7	108
		67 - 2	36	519	104				
High/Btm		67 - 3	37	551	110				
		67 - 4	38	549	110				

402028Quad.xls 3H
Printed: 11Jan2012 1:25 PM

Table 9. 15-Day Room Temperature Resuspension Homogeneity Assessment of the 9 August 2011 Formulations
(Analyzed 24 August 2011)

Group/ Strata	Dose Conc. (mg/mL)	Ref # (402028 -)	Line # (402028j-)	Analyzed Conc. (mg/mL)	Percent of Target (%)	Mean Conc. (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)
Low/Top	50	83 - 1	37	39.2	78.5	42.2	3.0	7.0	84.5
		83 - 2	38	46.3	92.6				
Low/Btm		83 - 3	39	41.7	83.4				
		83 - 4	40	41.7	83.4				
High/Top	500	84 - 1	41	439	87.9	439	21	4.8	87.7
		84 - 2	42	442	88.5				
High/Btm		84 - 3	43	411	82.3				
		84 - 4	44	462	92.4				

402028Quad.xls 4H
Printed: 11Jan2012 1:25 PM

Table 10. 8-Day Room Temperature Stability Assessment of the 9 August 2011 Formulations
(Analyzed 17-18 August 2011)

Strata	Dose Conc (mg/mL)	Ref # (402028 -)	Line # (402028i-)	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)	Percent of Time Zero (%)
Mid	50	54 - 1	40	47.8	95.5	51.8	5.7	11	104	95.6
		54 - 2	41	55.8	112					
Mid	500	65 - 1	42	495	98.9	521	36	7.0	104	96.9
		65 - 2	43	546	109					

Theoretical Conc (mg/mL)	Mean Time Zero Conc (mg/mL)
50	54.2
500	537

402028Quad.xls 3S8d(rt)
Printed: 11Jan2012 1:25 PM

Table 11. Mimimum 15-Day Room Temperature Stability Assessment of the 9 August 2011 Formulations
(Analyzed 24-25 August 2011)

Strata	Dose Conc (mg/mL)	Ref # (402028 -)	Line # (402028j-)	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)	Percent of Time Zero (%)
Mid	50	54 -3^	32	41.8	83.6	41.1	2.5	6.1	82.2	75.9
		54 -4^	33	38.3	76.6					
		54 -5*	50	40.1	80.2					
		54 -6*	51	44.2	88.3					
Mid	500	82 -1^	34	449	89.8	413	33	7.9	82.7	77.0
		82 -2^	35	413	82.7					
		86 -1*	52	421	84.2					
		86 -2*	53	370	74.1					

^Samples analyzed on 24 August 2011
*Samples analyzed on 25 August 2011

Theoretical Conc (mg/mL)	Mean Time Zero Conc (mg/mL)
50	54.2
500	537

402028Quad.xls 5S15d(rt)
Printed: 11Jan2012 1:25 PM

APPENDIX A

Study Protocol and Deviations

DEVIATIONS FROM THE PROTOCOL

This study was conducted in accordance with the protocol and protocol amendments, except for the following.

- **Protocol Section 6.3.4** states that processed sample stability will be assessed for the highest and lowest standard concentrations used on study. The processed sample stability assessment conducted on 18 August 2011 assessed stability of the 25.1 mg/mL standard and not the 10.0 mg/mL standard, which was the lowest calibration standard level.

  Reason for Deviation: Technician error.

  Impact: None

- **Protocol Section 6.3.5** states that samples collected for the assessment of resuspension homogeneity are to be collected in duplicate. Resuspension homogeneity samples collected on 17 August 2011 and 24 August 2011 were collected in quadruplicate, with 1 set of duplicate samples serving as samples to be analyzed and the second duplicate set serving as back-up samples.

  Reason for Deviation: Technician error.

  Impact: None

These deviations did not negatively impact the quality or integrity of the data nor the outcome of the study.

Study Number: WIL-402028

PROTOCOL AMENDMENT 1

Sponsor: American Petroleum Institute

Title of Study:

Analytical Validation and Stability Study of Ultra-Low Sulfur Diesel in Mineral Oil Formulations

Protocol Modifications:

1) **4.1.4 Lot Number:**

   A lot number will not be reported.

2) **4.1.5 Expiration/Retest Date:**

   An expiration/retest date will not be reported.

3) **4.1.6 Purity:**

   Purity value is not available for this material.

Reasons for Protocol Modification:

1) The barcode number (187840) was not provided on any of the samples received. Lack of lot number will be added to compliance section of report.

2) Documentation of the expiration/retest date for the test substance was not provided. Lack of information will be added to compliance section of report.

3) Due to the nature of the test substance (multiple components), a purity values is not applicable. Lack of information will be added to compliance section of report.

Approval:

Sponsor's approval was obtained via email on 13 Oct 2011.
Date

WIL Research Laboratories, LLC

Eric S. Bodle, PhD
Study Director
13 Oct 2011
Date

Farhad Sayyarpour, PhD
Director, Bioanalytical Chemistry
14 Oct 2011
Date

American Petroleum Institute

Russell White
Sponsor Representative
17 Oct 2011
Date

PROTOCOL

ANALYTICAL VALIDATION AND STABILTIY STUDY OF ULTRA-LOW SULFUR DIESEL IN MINERAL OIL 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 ultra-low sulfur diesel concentration in mineral oil formulations using gas chromatography (GC) with flame ionization or mass spectrometric detection. Mineral oil formulations prepared at test substance concentrations of 50 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:

Russell White
American Petroleum Institute
1220 L Street, NW
Washington, DC 20005
Tel: (202) 682-8344
Email: whiter@api.org

2.2 WIL Study Director:

Eric. S. Bodle, PhD
Assistant Director, Analytical Chemistry
Phone: (419) 289-8700
Fax: (419) 289-3650
E-mail: ebodle@wilresearch.com

2.3 WIL Departmental Responsibilities:

Robert E. Boes, MS
Associate Research Chemist, Analytical Chemistry
Emergency Contact
Tel: (419) 289-8700
Fax: (419) 289-3650
E-mail: rboes@wilresearch.com

Mark D. Nemec, BS, DABT
President and Chief Operating Officer

Michael J. Schlosser, PhD, DABT
Vice President, Analytical, Metabolism,
and *In Vitro* Toxicology Services

Heather L. Johnson, BS, RQAP-GLP
Manager, Quality Assurance

Robert A. Wally, BS
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:

Ultra-low sulfur diesel

4.1.2 CAS#:

68334-30-5

4.1.3 CAS definition:

A complex combination of hydrocarbons produced by the distillation of crude oil. It consists of hydrocarbons having carbon numbers predominantly in the range of C9 through C20 and boiling in the range of approximately 163°C to 357°C (325°F to 675°F).

4.1.4 Lot Number:

Blended ULSD (Barcode # 187840)

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:

- Mineral Oil with and without test substance

6 EXPERIMENTAL DESIGN:

6.1 Overview of the Study:

Ultra-low sulfur diesel 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 mineral oil 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:

Mineral Oil, USP (Spectrum Chemicals and Laboratory Products)

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 the analyte concentration in 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 (relative standard deviation [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 of Calibration Standards and Quality Control Samples:

The room temperature and/or autosampler temperature stability of calibration standards and processed quality control samples will be evaluated after a minimum of 24 hours of storage.

At least duplicate samples at the highest and lowest concentration levels evaluated will be analyzed post-storage, and the results will be compared to pre-storage values, results from analysis of freshly prepared samples, or the theoretical pre-storage values.

The analyte will be considered stable if the post-storage value is not less than 90% of the pre-storage (or pre-storage equivalent) value. If a >10% reduction occurs under the intended storage conditions, alternate storage conditions and/or durations may be evaluated as necessary to identify conditions that allow for stability during sample storage and processing.

6.3.5 Homogeneity, Resuspension Homogeneity, and Stability of Mineral Oil Formulations:

Test substance homogeneity, resuspension homogeneity, and stability in mineral oil formulations prepared at test substance concentrations of 50 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 at least 8 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.

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 e-mail on 15 Mar 2011.
Date

American Petroleum Institute

Russell White
Sponsor Representative

21-March-2011
Date

WIL Research Laboratories, LLC

Eric S. Bodle, PhD
Study Director

16 Mar 2011
Date

Michael J. Schlosser, PhD, DABT
Vice President,
Analytical, Metabolism, and *In Vitro* Toxicology Services

18 March 2011
Date