charles river

FINAL BIOANALYTICAL REPORT

Test Site Study No. 516655

Clinical Trial Reference No. C30031

Determination of HFPO-DA in EDTA Human Plasma Samples

SPONSOR:
The Chemours Company
1007 Market Street
Wilmington,
DE 19899
USA

TEST SITE:
Charles River Laboratories Den Bosch BV
Hambakenwetering 7
5231 DD ‘s-Hertogenbosch
The Netherlands

Charles River Laboratories Den Bosch BV
Nistelrooisebaan 3
5374 RE Schaijk
The Netherlands

TABLE OF CONTENTS

LIST OF TABLES 3
LIST OF FIGURES 3
LIST OF APPENDICES 3
COMPLIANCE STATEMENT 4
QUALITY ASSURANCE STATEMENT 5
1. RESPONSIBLE PERSONNEL 6
1.1. Test Site 6
1.2. Sponsor 6
2. SUMMARY 7
3. INTRODUCTION 8
4. MATERIALS AND METHODS 9
4.1. General 9
4.2. Test Item, Internal Standard and Matrix 9
4.2.1. Test Item 9
4.2.2. Internal Standard 9
4.2.3. Blank Matrix 10
4.3. Test Item and Internal Standard Characterization 10
4.4. Reserve Samples 10
4.5. Test Item and Internal Standard Inventory and Disposition 10
4.6. Chemicals and Reagents 11
4.7. Methods 11
4.7.1. Bioanalytical Method 11
4.7.2. Study Samples 11
4.7.3. System Suitability 11
5. CONSTRUCTED VARIABLES 12
6. COMPUTERIZED SYSTEMS 12
7. RETENTION OF RECORDS 12
8. RESULTS 13
8.1. Analytical Run History 13
8.2. Carry-over 13
8.3. Calibration Curves 13
8.4. QC Samples 13
8.5. Study Samples 13
8.6. Incurred Samples Reanalysis 14
9. CONCLUSION 14

LIST OF TABLES

Table 1 Analytical Run History 15
Table 2 Carry-over Percentages 16
Table 3 Calibration Curves 17
Table 4 QC Samples 18
Table 5 Study Samples 19
Table 6 Incurred Sample Reanalysis 20

LIST OF FIGURES

Figure 1 Representative Chromatograms and Calibration Curve 21

LIST OF APPENDICES

Appendix 1 Phase Study Plan, Amendments, and Deviations 32
Appendix 2 Test Item Characterization 53
Appendix 3 Internal Standard Characterization 55
Appendix 4 Bioanalytical Method 58

COMPLIANCE STATEMENT

The bioanalytical study was performed in accordance with the OECD Principles of Good Laboratory Practice as accepted by Regulatory Authorities throughout the European Union, United States of America, Japan, and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

Exceptions from the above regulations are listed below.

- Analyses conducted to support the information cited in the Certificate of Analysis or equivalent document for the test item and internal standard were not conducted in compliance with the GLP or Good Manufacturing Practice (GMP) regulations. The characterization of the test item was conducted under the suppliers quality system.

This study was conducted in accordance with the procedures described herein. All deviations authorized/acknowledged by the Study Director are documented in the Study Records. The report represents an accurate and complete record of the results obtained.

There were no deviations from the above regulations that affected the overall integrity of the study or the interpretation of the study results and conclusions.

T.P. Schoutsen Date: 30 October 2017.

T.P. Schoutsen, MSc.

Study Director

QUALITY ASSURANCE STATEMENT

Study title: Determination of HFPO-DA in EDTA human plasma samples.

This bioanalytical report was inspected by the Test Site Quality Assurance Unit (QAU) according to the Standard Operating Procedure(s). The reported method and procedures were found to describe those used and the report reflects the raw data. The Test Site inspection program was conducted in accordance with Standard Operating Procedure. During the on-site process inspections, procedures applicable to this type of study were inspected.

The dates of Quality Assurance inspections are given below.

Project 516655

Type of Inspections	Phase/Process	Start Inspection date	End Inspection date	Reporting date to TFM and SD*
Study	Study Plan	27-Jan-2017	27-Jan-2017	27-Jan-2017
	Study Plan Amendment 01	16-Feb-2017	16-Feb-2017	16-Feb-2017
	Report	05-Sep-2017	07-Sep-2017	07-Sep-2017
Process	**Test Substance Receipt** Test Item Handling	06-Feb-2017	09-Feb-2017	13-Feb-2017
	Bioanalysis Test Item Handling Observations/Measurements Specimen Handling	06-Feb-2017	17-Feb-2017	22-Feb-2017

*TFM=Test Facility Management SD = Study Director

The review of the final report was completed on the date of signing this QA statement.

Date: 27 October 2017

Marjolijn Kock, MSc
Quality Assurance Auditor

1. RESPONSIBLE PERSONNEL

1.1. Test Site

Study Director (Analytical Project Manager)	T.P. Schoutsen, MSc.
Test Site Management	E. Baltussen, PhD.

1.2. Sponsor

Sponsor Representative / Study Monitor	S. Gannon, Ph.D., DABT

2. SUMMARY

The objective of the bioanalytical study was to analyze study samples of EDTA human plasma for the determination of HFPO-DA.

The summary of the bioanalytical study is given below.

Analyte	HFPO-DA
Internal standard	M3HFPO-DA
Matrix	human plasma
Anticoagulant	K_2-EDTA
Detector	AB Sciex API4000

Information			**Hyperlink**
Analytical run history			Table 1
Carry-over	≤ 20% of the LLOQ for the analyte ≤ 5% of the LLOQ for the internal standard		Table 2
Calibration curves	1.00 - 500 ng/mL		Table 3
QC samples	QC-low (3 ng/mL)	85-115%	Table 4
	QC-middle (25 ng/mL)	85-115%	
	QC-high (375 ng/mL)	85-115%	
Study samples			Table 5
Incurred sample reanalysis (ISR)	≥ 67% of samples acceptable with deviation of ≤ 20%		Table 6

In conclusion, the reported results are considered representative of the concentration of HFPO-DA in human plasma for this study.

3. INTRODUCTION

The objective of the bioanalytical study was to analyze study samples of EDTA human plasma for the determination of HFPO-DA.

The design of this study is based on the following study guidelines:

- European Medicines Agency (EMA). *Guideline on Bioanalytical Method Validation*. EMEA/CHMP/EWP/192217/2009. 21 July 2011.
- FDA Guidance for Industry: *Bioanalytical Method Validation*. May 2001.
- EMA *Reflection Paper for Laboratories that Perform the Analysis or Evaluation of Clinical Trial Samples*. EMA/INS/GCP/532137/2010. 28 February 2012.
- ICH Harmonised Tripartite Guideline E6(R1). *Guideline for Good Clinical Practice*. 10 June 1996.
- UK Medicines and Healthcare Products Regulatory Agency (MHRA). *Good Clinical Practice Guidance on the Maintenance of Regulatory Compliance in Laboratories that Perform the Analysis or Evaluation of Clinical Trial Samples*. July 2009.

For the work detailed in this phase report, the bioanalytical phase start date was 29 Mar 2017, and the bioanalytical phase completion date was 21 Apr 2017. The phase study plan, study plan amendments, and deviations are presented in Appendix 1.

The bioanalytical study was performed at the Schaijk location, all other supporting activities were performed at the ‘s-Hertogenbosch location.

4. MATERIALS AND METHODS

4.1. General

General data concerning chemicals, reagents, preparation of stock solutions, calibration standards, and quality control samples (QCs) as well as the analytical conditions were stored under project number 516652.

4.2. Test Item, Internal Standard and Matrix

4.2.1. Test Item

Identification:	HFPO-DA
Appearance:	Colourless liquid (determined by Charles River Den Bosch)
Batch:	HFPODA0616
Purity/Composition:	50 ± 2.5 µg/mL in methanol
Test item storage:	In refrigerator (2-8°C)
Stable under storage conditions until:	25 June 2019 (expiry date)
Supplier:	Wellington Laboratories Inc., Ontario, Canada
Additional information	
Test Site test item number:	208124/A
Purity/Composition correction factor:	No correction factor required
Test item handling:	No specific handling conditions required

4.2.2. Internal Standard

Identification:	M3HFPO-DA
Appearance:	Colourless liquid (determined by Charles River Den Bosch)
Batch:	M3HFPODA0213
Purity/Composition:	50 ± 2.5 µg/mL in methanol
Chemical Purity	>98%
Isotopic purity	≥99% $^{13}C(^{13}C_3)$
Storage:	in the refrigerator (2-8°C)
Stable under storage conditions until:	21 July 2018 (expiry date)
Supplier:	Wellington Laboratories Inc., Ontario, Canada
Additional information	
Test Facility analytical standard number:	AS1673/A

Identification:	M3HFPO-DA
Appearance:	Colourless liquid (determined by Charles River Den Bosch)
Batch:	M3HFPODA0616
Purity/Composition:	50 ± 2.5 µg/mL in methanol
Chemical Purity	> 98%
Isotopic purity	≥ 99% $^{13}C(^{13}C_3)$
Storage:	in the refrigerator (2-8°C)
Stable under storage conditions until:	25 June 2019 (expiry date)
Supplier:	Wellington Laboratories Inc., Ontario, Canada

Additional information

Test Facility analytical standard number:	AS1673/B

Additional information for both batches

Purity/Composition correction factor:	No correction factor required
Analytical standard handling:	No specific handling conditions required

4.2.3. Blank Matrix

Blank human plasma with K_2-EDTA as anticoagulant was obtained from Sera Laboratories International (Sera Lab, Burgess Hill, West Sussex, UK). Information on the gender is given below. Blank matrices were stored in the ultra-low freezer (≤ -75°C).

Text Table 1
Blank Matrix

Type of matrix	Gender	Hemolytic	Supplier
Plasma	Mix male/female	No	Sera Lab

4.3. Test Item and Internal Standard Characterization

The supplier provided to the Test Facility documentation of the identity, purity, composition, and stability for the test item and internal standard. Certificates of Analysis or equivalent documents were provided to the Test Facility and are presented in Appendix 2 and Appendix 3.

4.4. Reserve Samples

A reserve sample of the test item analytical standard was not collected and maintained due to the limited amount available.

4.5. Test Item and Internal Standard Inventory and Disposition

Records of the receipt, distribution, and storage of test item and internal standard were maintained.

4.6. Chemicals and Reagents

Text Table 2
Chemicals and Reagents

Chemical / Reagent	Grade	Supplier
Water	Tap water purified by a Milli-Q water purification system	Millipore, Bedford, MA, USA
Acetonitrile	ULC/MS-CC/SFC	Biosolve, Valkenswaard, The Netherlands
Methanol	ULC/MS-CC/SFC	Biosolve
Ammonium formate	ULC/MS-CC/SFC	Biosolve
Formic acid	ULC/MS-CC/SFC 99%	Biosolve
2-Propanol	LiChrosolv	Merck Darmstadt, Germany

4.7. Methods

4.7.1. Bioanalytical Method

Quantitative analysis was based on the bioanalytical method validated for the analyte in EDTA human plasma in project 516654.

The analytical conditions, preparation of solutions and sample processing method are given in Appendix 4.

4.7.2. Study Samples

A total of 24 study samples in polypropylene tubes were received as described below, and were stored in the ultra-low freezer (≤ -75°C) until analysis.

Text Table 3
Study Samples

Date of delivery	Number of samples	Conditions on receipt
06 March 2017	5	Frozen (cooling element)
08 March 2017	5	Frozen (cooling element)
10 March 2017	4 (in duplicates)	Frozen (Dry ice)
14 March 2017	2 (in duplicates)	Frozen (Dry ice)
20 March 2017	2	Frozen (cooling element)
22 March 2017	4	Frozen (Dry ice)
27 March 2017	2	Frozen (cooling element)

Study samples were thawed at room temperature. Sample processing was performed according to the sample processing method in Appendix 4.

All study samples were analyzed within the proven stability period of 29 days.

4.7.3. System Suitability

On each day of analysis, a system suitability standard was injected in triplicate before analytical batches were submitted.

The system suitability standards had retention times and peak height/peak area ratios of the analyte and internal standard that were within acceptance ranges (see Appendix 4). It demonstrated that the LC-MS/MS instrument was suitable for the analysis of the analyte and internal standard on the day of analysis.

5. CONSTRUCTED VARIABLES

Response: Peak area of the analyte or internal standard

Response ratio (R):

$$\frac{\text{Peak area of analyte}}{\text{Peak area of internal standard}}$$

Calibration curve:

$$R = a\,C_N^2 + b\,C_N + c$$

where:
C_N nominal concentration
a quadratic regression factor
b linear regression factor
c intercept

Analyzed concentration (CA):

$$C_A = \frac{-b + \sqrt{b^2 - 4a \times (c - R)}}{2a} \times d$$

where:
d dilution factor

Accuracy:

$$\frac{C_A}{C_N} \times 100 \quad [\%]$$

Difference:

$$\frac{\text{highest value - lowest value}}{\text{mean value}} \times 100 \quad [\%]$$

All results presented in the tables of the report are calculated using values as per the raw data rounding procedure and may not be exactly reproduced from the individual data presented.

6. COMPUTERIZED SYSTEMS

Critical computerized systems used in the phase are listed below. All computerized systems used in the conduct of this phase have been validated.

Text Table 4
Critical Computerized Systems

System name	Version No.	Description of Data Collected and/or Analyzed
Analyst	1.6.2	System control, data acquisition and processing
LabWare LIMS	5.02m+	Laboratory information management system (LIMS)
REES Centron	SQL 2.0	Temperature, relative humidity and/or atmospheric pressure monitoring

7. RETENTION OF RECORDS

All phase-specific raw data, electronic data, documentation (e.g. relevant emails), phase study plan, and final phase report were archived at the Test Site by no later than the date of final phase report issue unless otherwise specified in the study plan. At least five years after issue of the final phase report, the Sponsor will be contacted.

8. RESULTS

Representative chromatograms and a calibration curve are presented in Figure 1.

8.1. Analytical Run History

The analytical run history of the project is given in Table 1.

8.2. Carry-over

Double blank samples were analyzed after each highest calibration standard to evaluate the carry-over of the analytical system. The carry-over percentages are given in Table 2.

No carry-over at the retention time of the analyte and internal standard was observed.

8.3. Calibration Curves

Calibration standards at each concentration level were injected in duplicate. One replicate was analyzed at the beginning and one at the end of the analytical run. A double blank sample and a single blank sample were analyzed before the first set of calibration standards. The response percentages of the analyte and internal standard in the blank samples to the lowest calibration standard are given in Table 3.

Calibration curves in the concentration range of 1.00 - 500 ng/mL for the analyte in human plasma were obtained by quadratic regression analysis with a $1/x^2$ weighting factor. Since the back calculated accuracies of the data points were in the range 80-120% for the lowest calibration standard and 85-115% for the remaining calibration standards the calibration curves were accepted. The individual results and statistical parameters of the calibration curves are given in Table 3.

8.4. QC Samples

QC-low, middle and high samples were analyzed in duplicate in each analytical run. The results of the QC samples are given in Table 4.

Since the accuracy of ≥ 2/3 of the QC samples and ≥ 50% per concentration level fell in the criterion range of 85-115% the analytical runs were accepted.

The overall mean accuracy and coefficient of variation of the QC samples at each concentration level fell within the criterion range of 85-115% and ≤ 15%, respectively.

8.5. Study Samples

The results of the study samples are given in Table 5.The results are presented in ng/mL used as original concentration and calculated to µg/mL by dividing the concentration by 1000 after a request of the sponsor.

8.6. Incurred Samples Reanalysis

The results of the incurred samples are given in Table 6.

Most of the samples had a concentration less than QC-L concentration level so ISR on a minimum of 20 study samples with a preferred concentration above 3 time the LLOQ was not possible. Therefore, a total of 5 incurred samples were subjected to reanalysis. The difference between the analyzed concentrations for the initial and reanalysis was ≤ 20% for 100% of the mean repeats. The criterion range of at least 67% for incurred sample reanalysis (ISR) was met, demonstrating that the analytical method was reliable.

The analyzed concentrations of the incurred samples were reported in a separate table and were not used for toxicokinetic purposes. ISR only relates to the evaluation of analytical method performance.

9. CONCLUSION

In conclusion, the reported results are considered representative of the concentration of analyte in human plasma for this study.

Table 1
Analytical Run History

Project: 516655

Validated report name: Analytical run history
Report version: 3.1

Analytical run history of TI208124

Analytical run	Date of analysis	Approval	Remarks
SA_001	29-Mar-2017	Accepted	None
SA_002	31-Mar-2017	Rejected	Rejection due to accuracy of QC samples; < 2/3 of the QC samples were within the acceptance criteria.
SA_003	03-Apr-2017	Rejected	Rejection due to accuracy of QC samples; both accuracies of one QC level were outside the acceptance criteria.
SA_004	20-Apr-2017	Rejected	Rejection due to accuracy of QC samples; both accuracies of one QC level were outside the acceptance criteria.
SA_005	21-Apr-2017	Accepted	None

Bram van Gemert
08-May-2017

Table 2
Carry-over Percentages

Project: 516655

Validated report name: Carry-over

Report version: 3.0

Carry-over percentages of TI208124 in double blank matrix

Analytical run	Carry-over	
	I	II
SA_001	0	0
SA_005	0	0

Project: 516655

Validated report name: Carry-over

Report version: 3.0

Carry-over percentages of internal standard for TI208124 in double blank matrix

Analytical run	Carry-over	
	I	II
SA_001	0	0
SA_005	0	0

Table 3
Calibration Curves

Project: 516655

Validated report name: Calibration blanks
Report version: 3.0

Calibration blanks TI208124

	Double blank [%]	Blank [%]
SA_001	0	0
SA_005	0	0

Project: 516655

Validated report name: Calibration results PT
Report version: Version 3.2

Back calculated concentrations [ng/mL] and accuracy [%] of TI208124

Analytical run	1.00	2.50	7.50	15.0	35.0	100	200	500
SA_001	0.951	2.68	7.34	15.1	32.6	104	203	485
	95%	107%	98%	101%	93%	104%	101%	97%
	1.02	2.59	7.39	13.9	34.5	100	220	492
	102%	104%	99%	93%	99%	100%	110%	98%
SA_005	0.902	2.66	7.83	16.6	32.0	94.6	195	522
	90%	106%	104%	111%	91%	95%	97%	104%
	1.04	2.65	7.69	15.5	32.8	92.6	199	499
	104%	106%	103%	103%	94%	93%	100%	100%

Project: 516655

Validated report name: Calibration parameters Quadratic
Report version: 3.1

Statistical parameters TI208124

Analytical run	Quadratic regression factor	Linear regression factor	Intercept
SA_001	-7.600E-07	3.539E-02	5.219E-03
SA_005	2.553E-06	3.311E-02	4.041E-03

Table 4
QC Samples

Project: 516655

Validated report name: Quality controls

Report version: 3.1

Individual data of the QC samples [ng/mL] for TI208124

Analytical run	QC-low 3.00		QC-middle 25.0		QC-high 375	
	1	2	1	2	1	2
SA_001	3.25 108%	3.40 113%	22.7 91%	25.1 100%	416 111%	437 116%
SA_005	3.41 114%	3.37 112%	25.6 103%	27.3 109%	410 109%	419 112%

Project: 516655

Validated report name: Quality controls

Report version: 3.1

Mean accuracy and precision of the QC samples for TI208124

	QC-low	QC-middle	QC-high
Overall accuracy [%]	112	101	112
Overall CV [%]	2	7	3

Table 5
Study Samples

Analyzed concentrations of TI208124 (ng/mL) in plasma samples of Group A with recalculation to µg/ml (= ppm) in plasma samples.

Session	Time point	Subject	ng/mL	µg/mL (= ppm)
Period 1	-	1	< 1.00	< 0.00100
		2	26.6	0.0266
		3	51.2	0.0512
		4	1.22	0.00122
		5	4.87	0.00487
		6	< 1.00	< 0.00100
		7	< 1.00	< 0.00100
		8	169	0.169
		9	1.77	0.00177
		10	1.20	0.00120
		11	1.82	0.00182
		12	6.11	0.00611
		13	1.70	0.00170
		14	1.38	0.00138
		15	2.71	0.00271
		16	< 1.00	< 0.00100
		17	2.48	0.00248
		18	< 1.00	< 0.00100
		19	< 1.00	< 0.00100
		20	2.85	0.00285
		21	1.23	0.00123
		22	1.00	0.00100
		23	< 1.00	< 0.00100
		24	2.87	0.00287

Table 6
Incurred Sample Reanalysis
Results in ng/mL

Project: 516655

Validated report name: ISR results
Report version: 3.0

Incurred sample reanalysis for TI208124

Study sample	Analyzed concentration (ng/mL)		Difference [%]
	Original	ISR	
gr.a-sj.12-per. 1-t=-	6.11	6.35	4
gr.a-sj.2-per. 1-t=-	26.6	23.4	13
gr.a-sj.3-per. 1-t=-	51.2	54.9	7
gr.a-sj.5-per. 1-t=-	4.87	5.11	5
gr.a-sj.8-per. 1-t=-	169	160	6

Number of ISR samples 5

Values of ≤ 20% of origin [%] 100

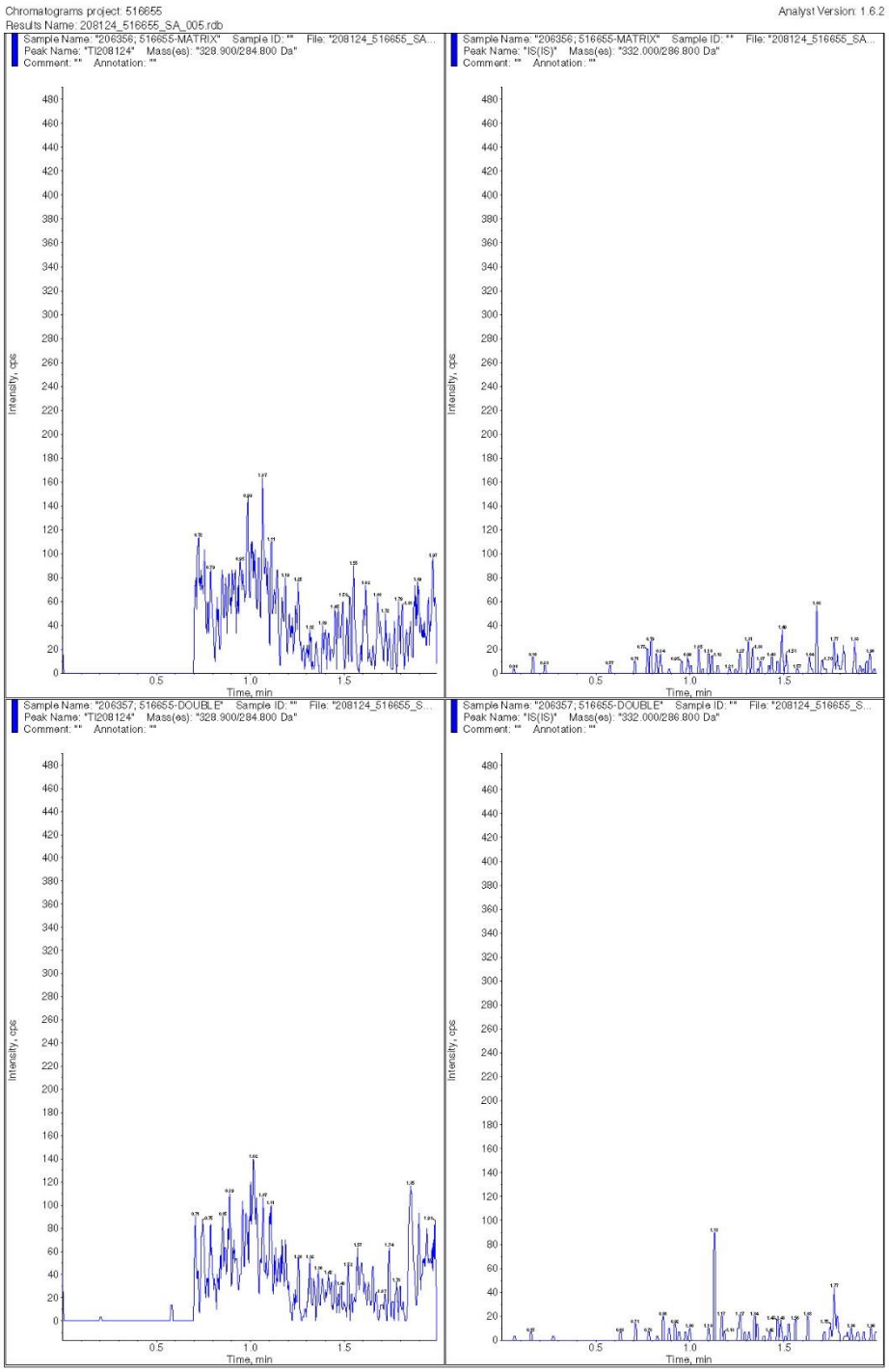
Results in µg/mL (=ppm)

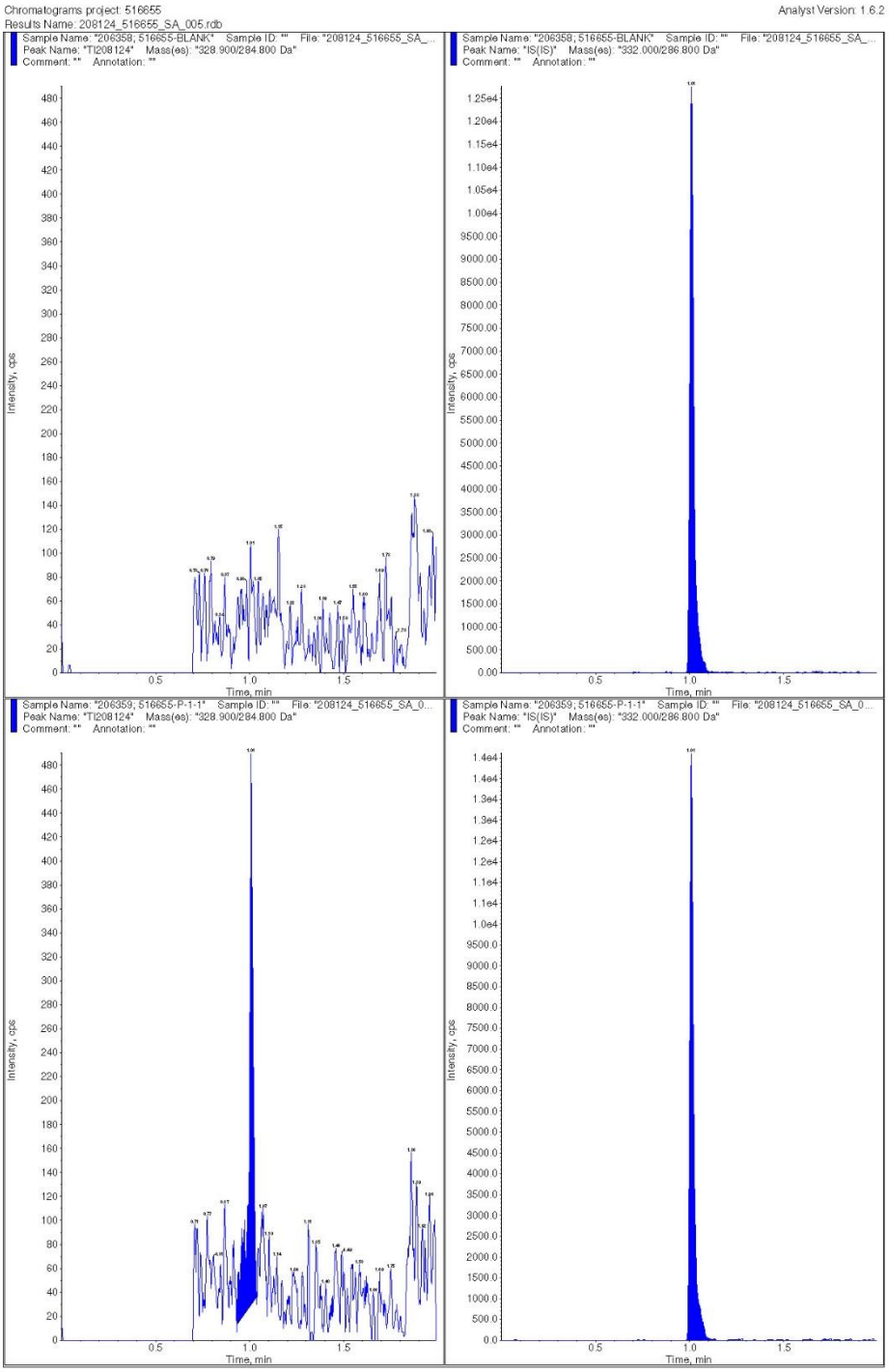
Study sample	Analyzed concentration (µg/mL)		Difference [%]
	Original	ISR	
gr.a-sj.12-per. 1-t=-	0.00611	0.00635	4
gr.a-sj.2-per. 1-t=-	0.0266	0.0234	13
gr.a-sj.3-per. 1-t=-	0.0512	0.0549	7
gr.a-sj.5-per. 1-t=-	0.00487	0.00511	5
gr.a-sj.8-per. 1-t=-	0.169	0.160	6

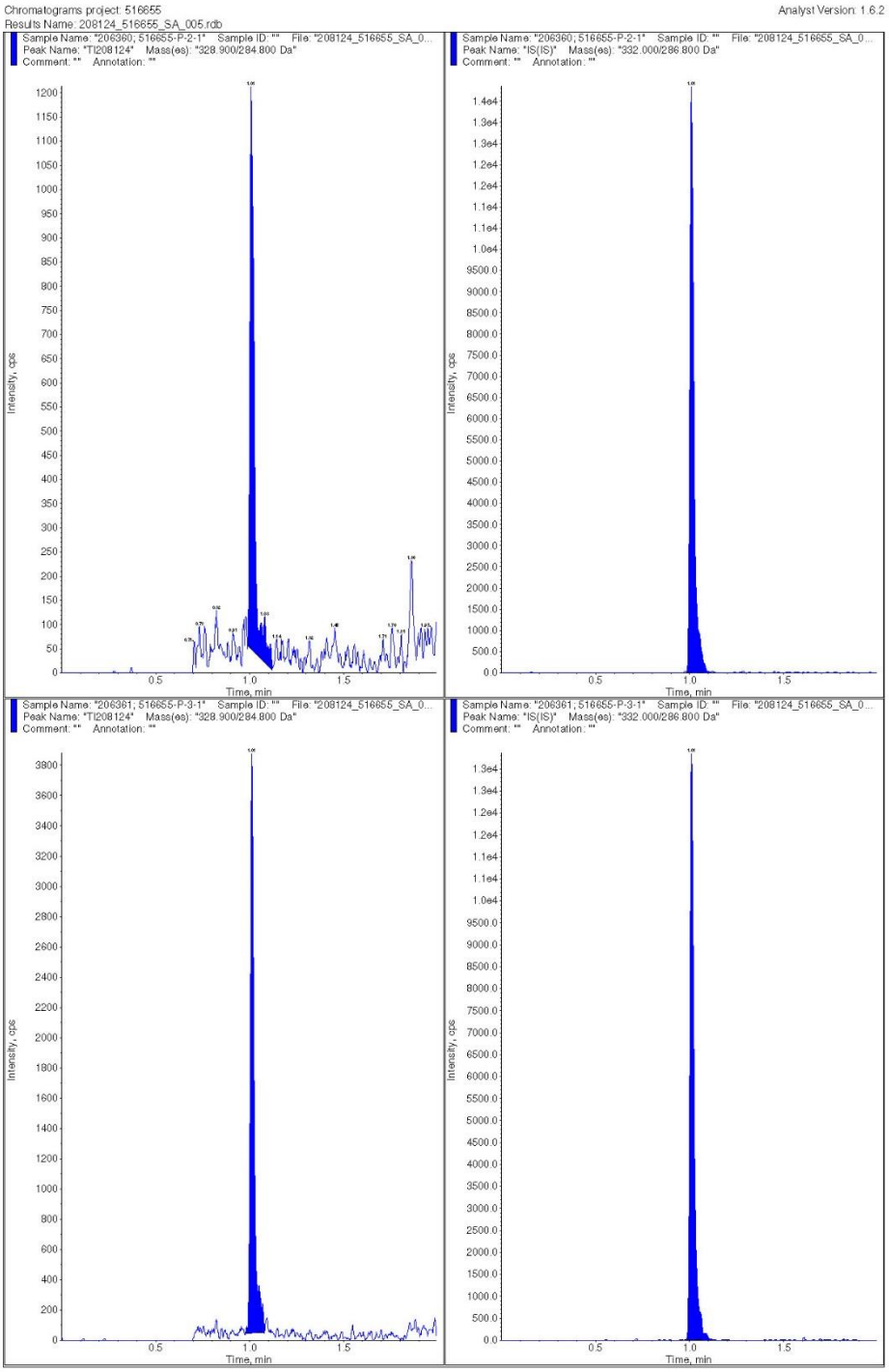
Number of ISR samples 5

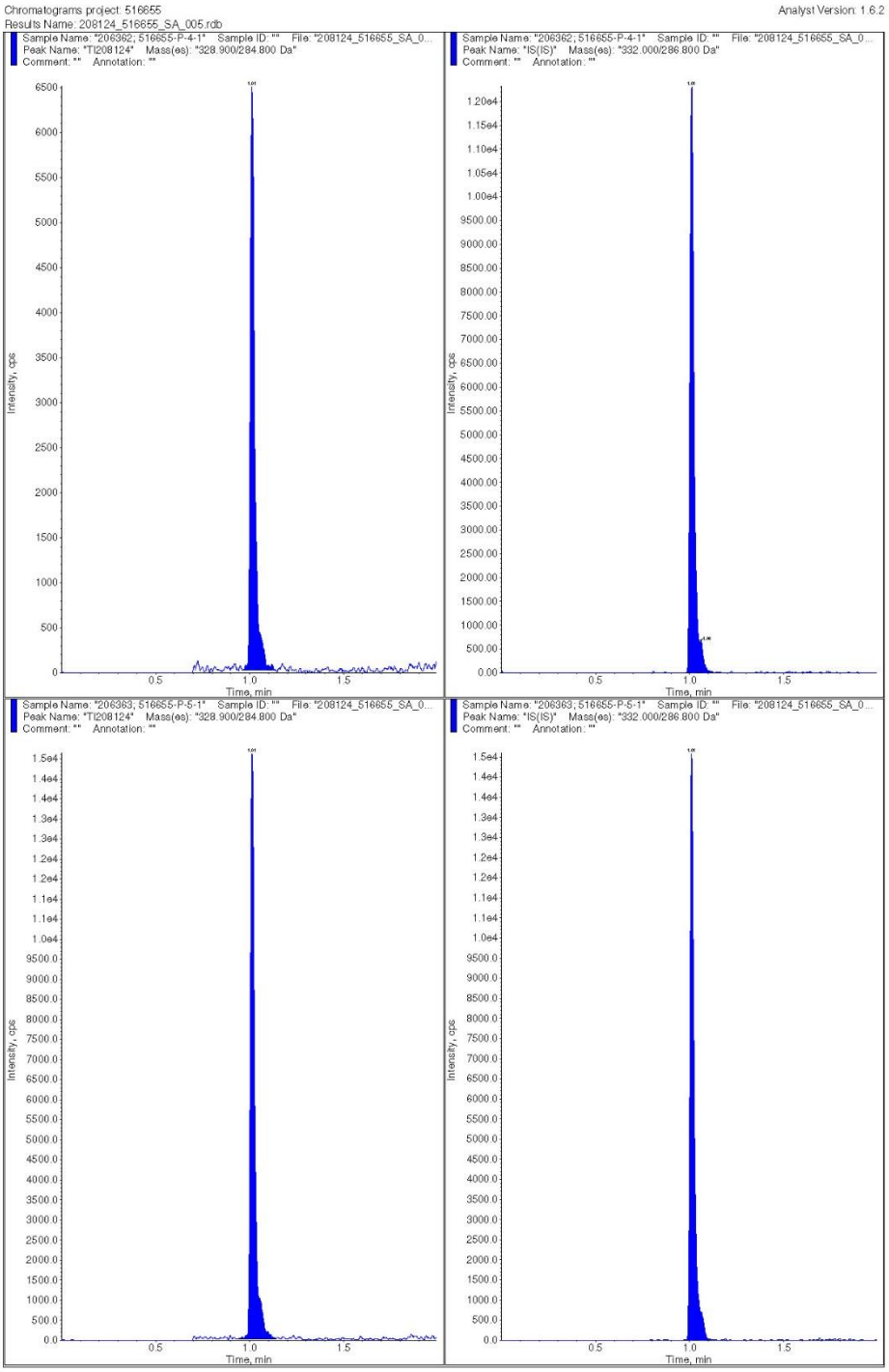
Values of ≤ 20% of origin [%] 100

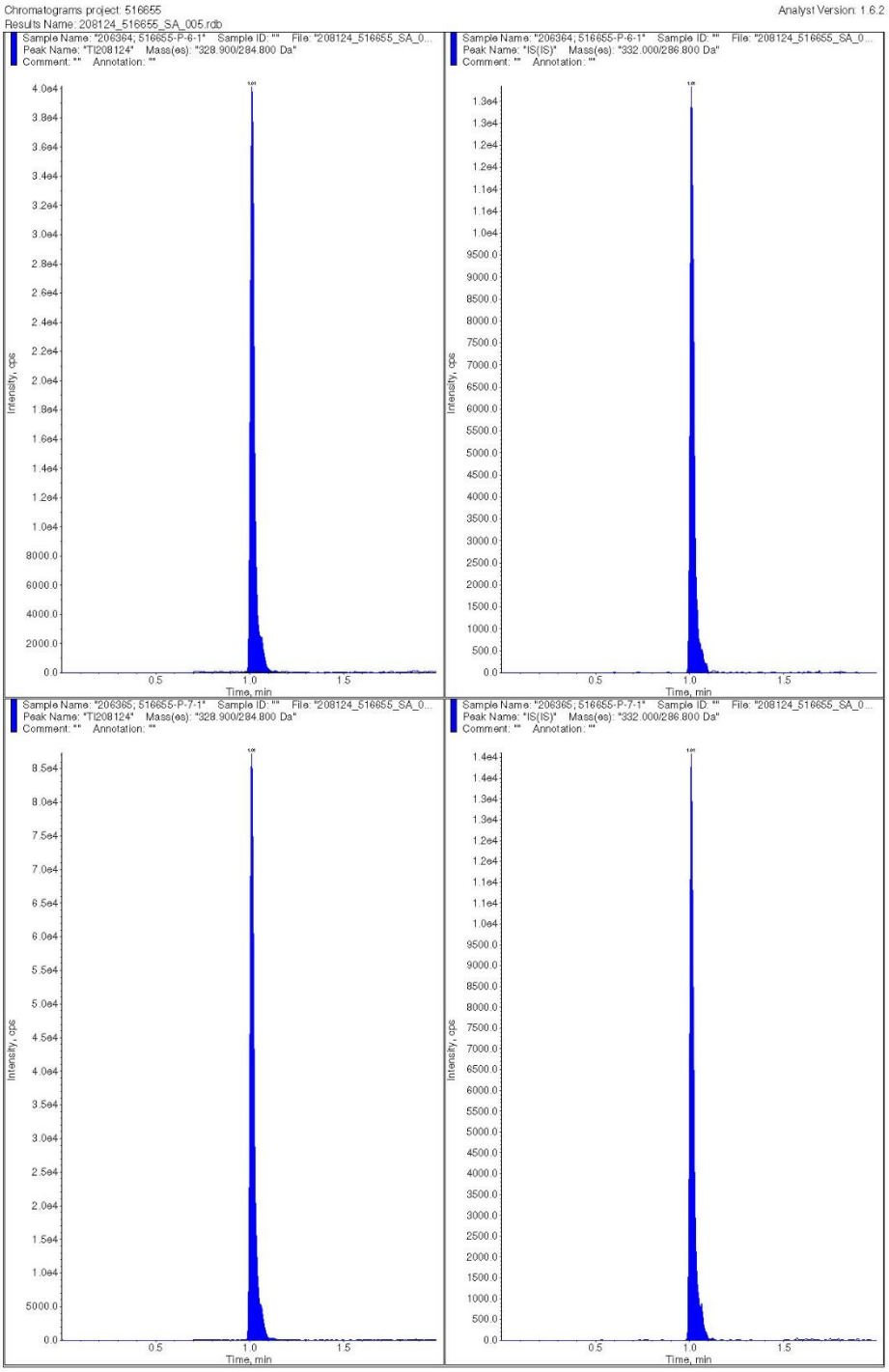
Figure 1
Representative Chromatograms and Calibration Curve

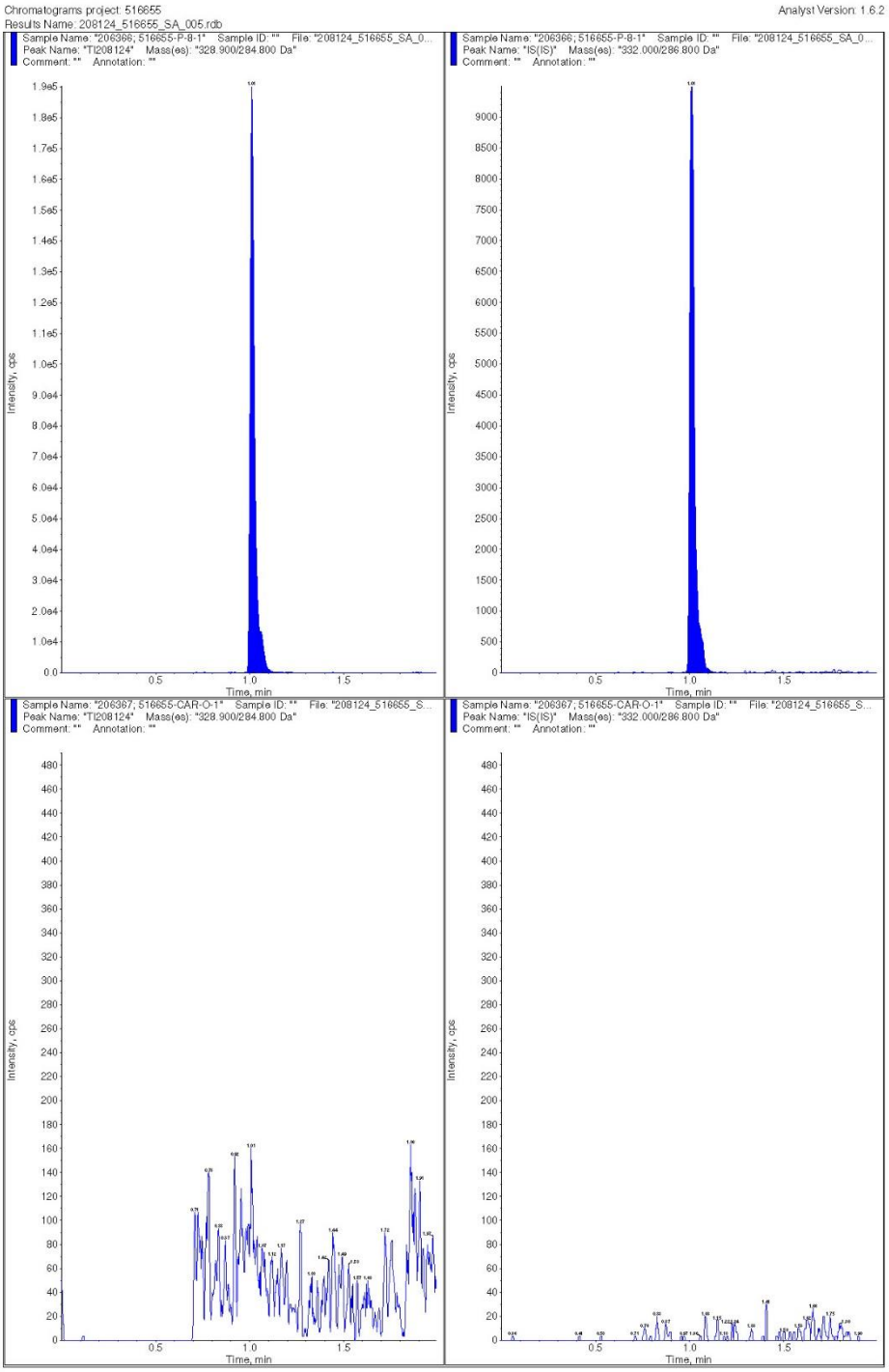


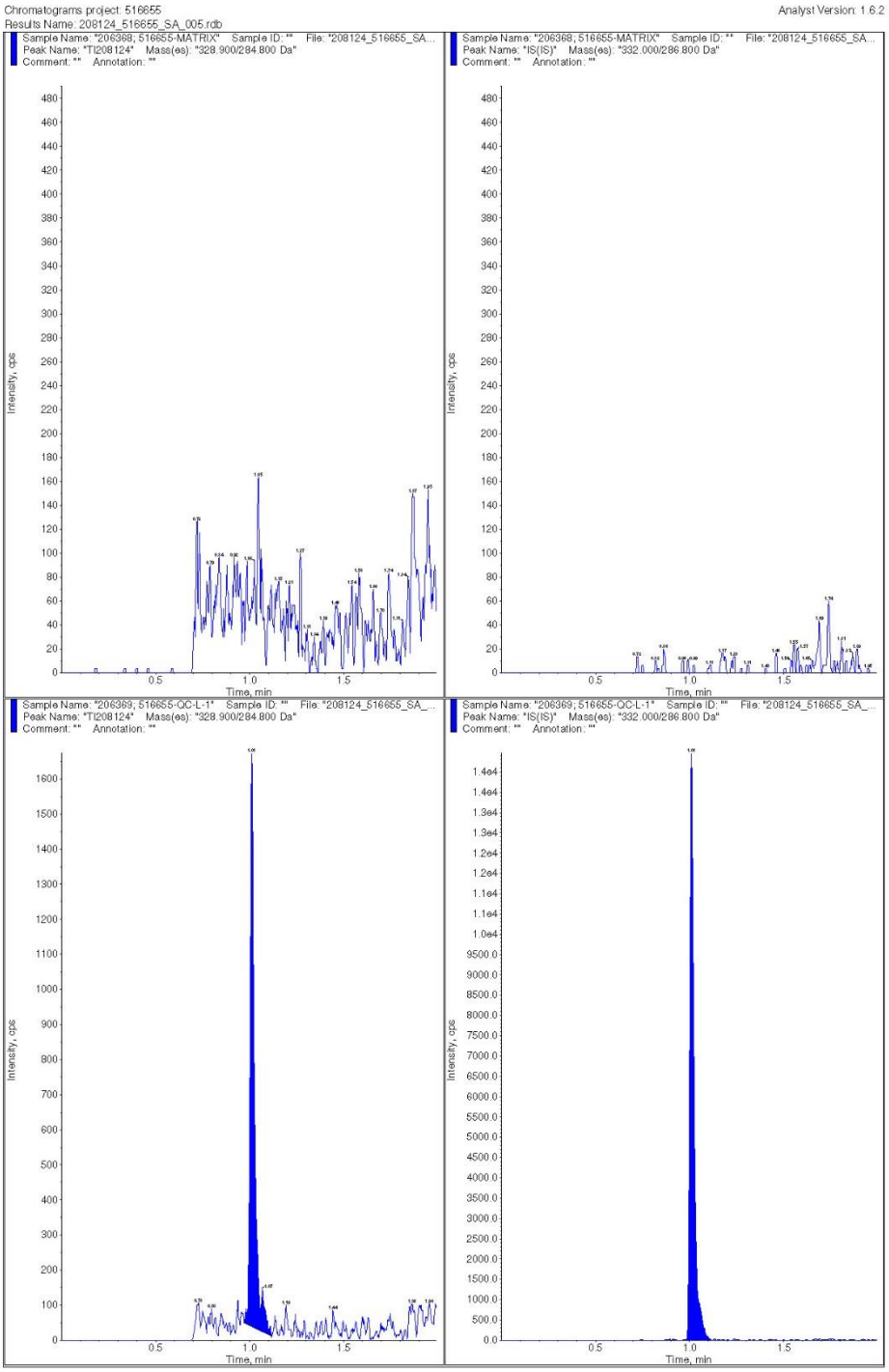

Chromatograms project: 516655
Analyst Version: 1.6.2
Results Name: 208124_516655_SA_005.rdb
Sample Name: "206358; 516655-BLANK" Sample ID: "" File: "208124_516655_SA_...
Peak Name: "TI208124" Mass(es): "328.900/284.800 Da"
Comment: "" Annotation: ""
Sample Name: "206358; 516655-BLANK" Sample ID: "" File: "208124_516655_SA_...
Peak Name: "IS(IS)" Mass(es): "332.000/286.800 Da"
Comment: "" Annotation: ""
Sample Name: "206359; 516655-P-1-1" Sample ID: "" File: "208124_516655_SA_0...
Peak Name: "TI208124" Mass(es): "328.900/284.800 Da"
Comment: "" Annotation: ""
Sample Name: "206359; 516655-P-1-1" Sample ID: "" File: "208124_516655_SA_0...
Peak Name: "IS(IS)" Mass(es): "332.000/286.800 Da"
Comment: "" Annotation: ""
Intensity, cps
Time, min

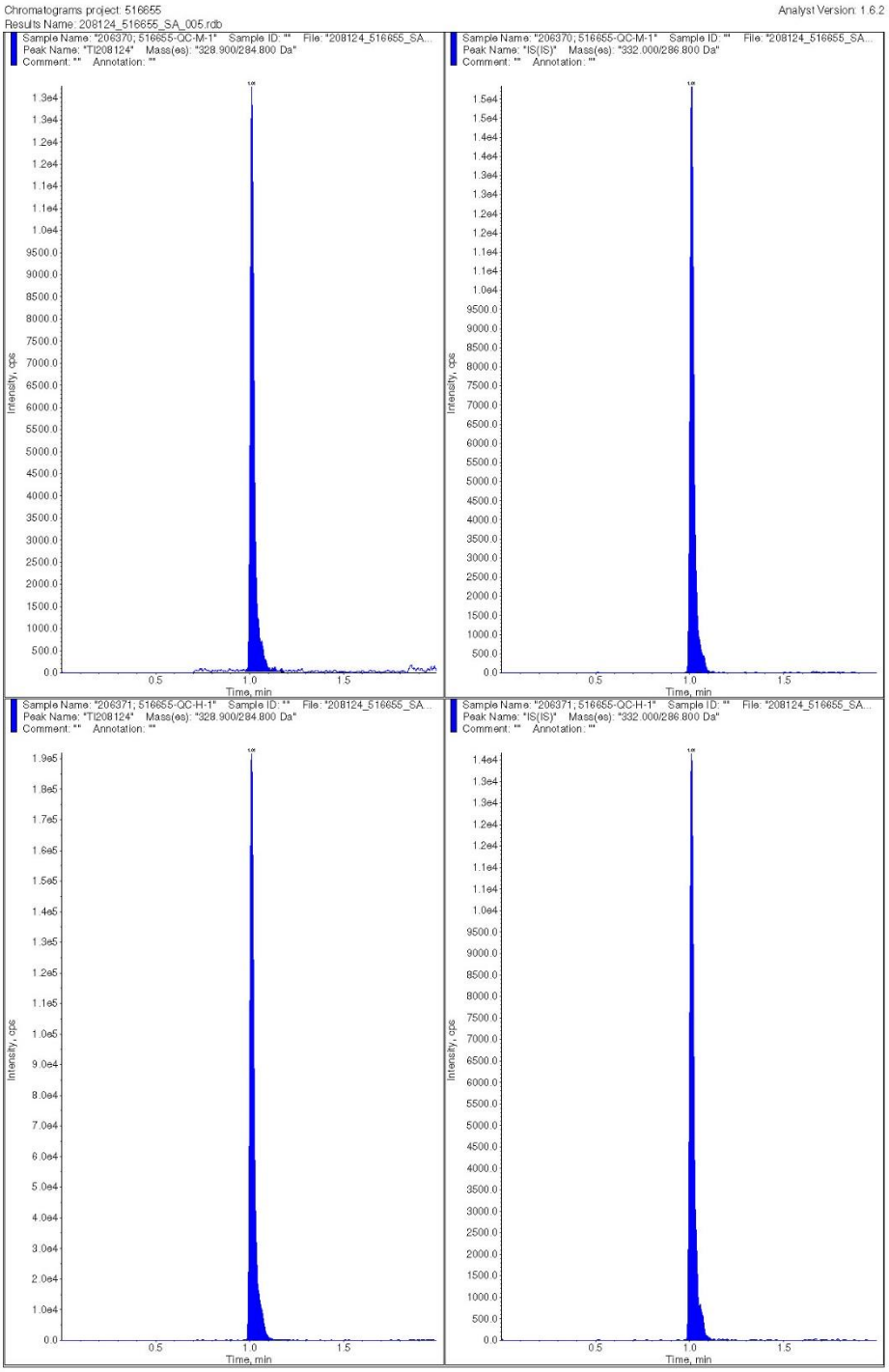

Chromatograms project: 516655
Analyst Version: 1.6.2
Results Name: 208124_516655_SA_005.rdb
Sample Name: "206360; 516655-P-2-1" Sample ID: "" File: "208124_516655_SA_0...
Peak Name: "TI208124" Mass(es): "328.900/284.800 Da"
Comment: "" Annotation: ""
Sample Name: "206360; 516655-P-2-1" Sample ID: "" File: "208124_516655_SA_0...
Peak Name: "IS(IS)" Mass(es): "332.000/286.800 Da"
Comment: "" Annotation: ""
Sample Name: "206361; 516655-P-3-1" Sample ID: "" File: "208124_516655_SA_0...
Peak Name: "TI208124" Mass(es): "328.900/284.800 Da"
Comment: "" Annotation: ""
Sample Name: "206361; 516655-P-3-1" Sample ID: "" File: "208124_516655_SA_0...
Peak Name: "IS(IS)" Mass(es): "332.000/286.800 Da"
Comment: "" Annotation: ""
Intensity, cps
Time, min

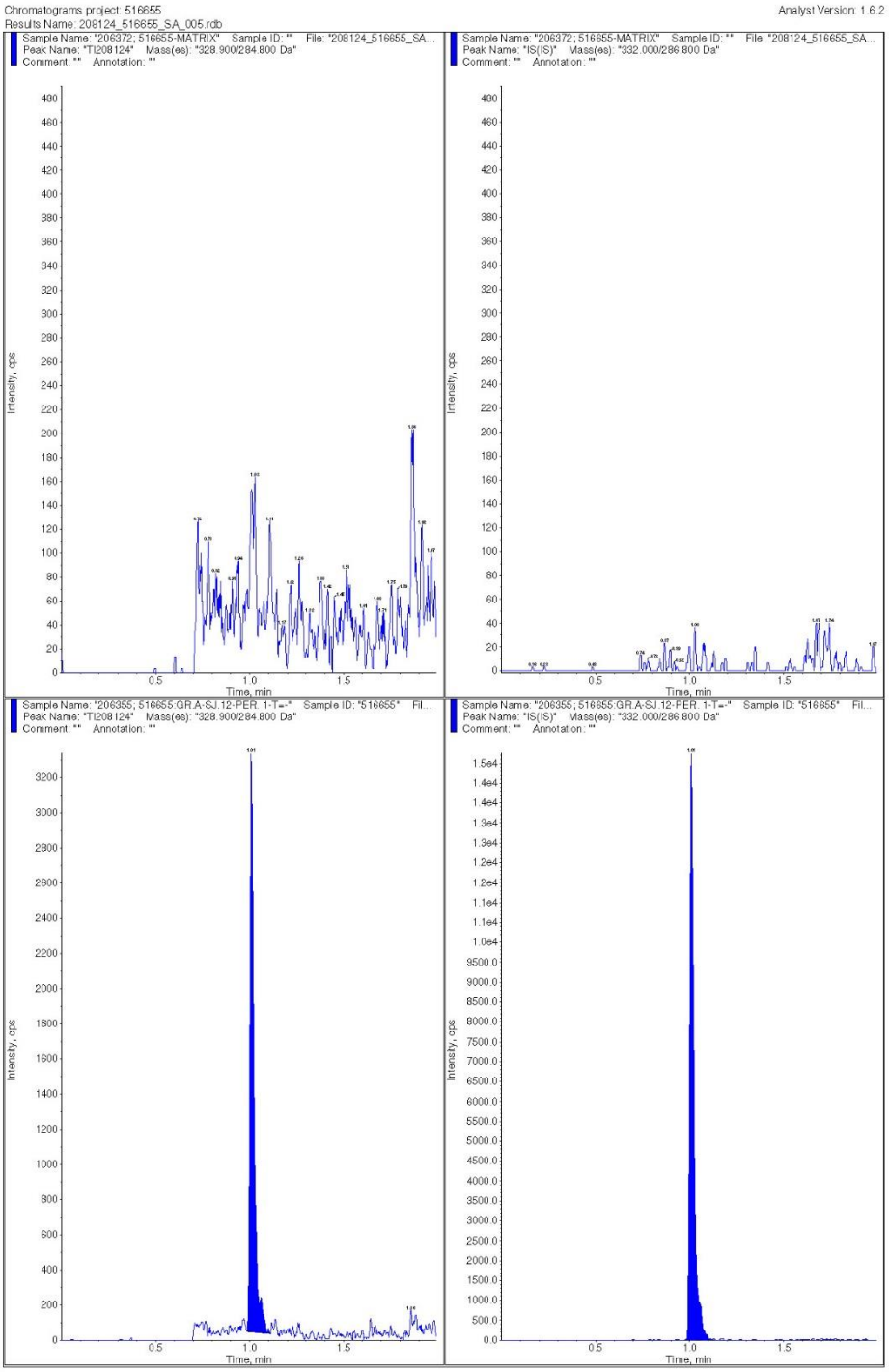

Chromatograms project: 516655
Results Name: 208124_516655_SA_005.rdb
Analyst Version: 1.6.2
Sample Name: "206362; 516655-P-4-1" Sample ID: "" File: "208124_516655_SA_0...
Peak Name: "TI208124" Mass(es): "328.900/284.800 Da"
Comment: "" Annotation: ""
Sample Name: "206362; 516655-P-4-1" Sample ID: "" File: "208124_516655_SA_0...
Peak Name: "IS(IS)" Mass(es): "332.000/286.800 Da"
Comment: "" Annotation: ""
Sample Name: "206363; 516655-P-5-1" Sample ID: "" File: "208124_516655_SA_0...
Peak Name: "TI208124" Mass(es): "328.900/284.800 Da"
Comment: "" Annotation: ""
Sample Name: "206363; 516655-P-5-1" Sample ID: "" File: "208124_516655_SA_0...
Peak Name: "IS(IS)" Mass(es): "332.000/286.800 Da"
Comment: "" Annotation: ""
Intensity, cps
Time, min

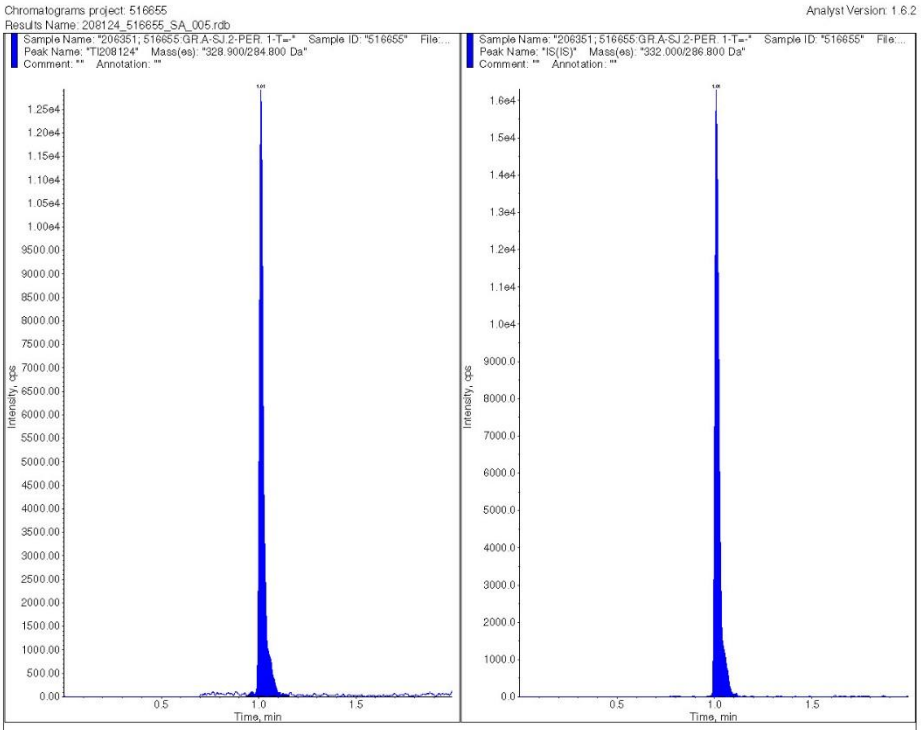

Chromatograms project: 516655
Analyst Version: 1.6.2
Results Name: 208124_516655_SA_005.rdb
Sample Name: "206364; 516655-P-6-1" Sample ID: "" File: "208124_516655_SA_0...
Peak Name: "TI208124" Mass(es): "328.900/284.800 Da"
Comment: "" Annotation: ""
Sample Name: "206364; 516655-P-6-1" Sample ID: "" File: "208124_516655_SA_0...
Peak Name: "IS(IS)" Mass(es): "332.000/286.800 Da"
Comment: "" Annotation: ""
Sample Name: "206365; 516655-P-7-1" Sample ID: "" File: "208124_516655_SA_0...
Peak Name: "TI208124" Mass(es): "328.900/284.800 Da"
Comment: "" Annotation: ""
Sample Name: "206365; 516655-P-7-1" Sample ID: "" File: "208124_516655_SA_0...
Peak Name: "IS(IS)" Mass(es): "332.000/286.800 Da"
Comment: "" Annotation: ""
Intensity, cps
Time, min

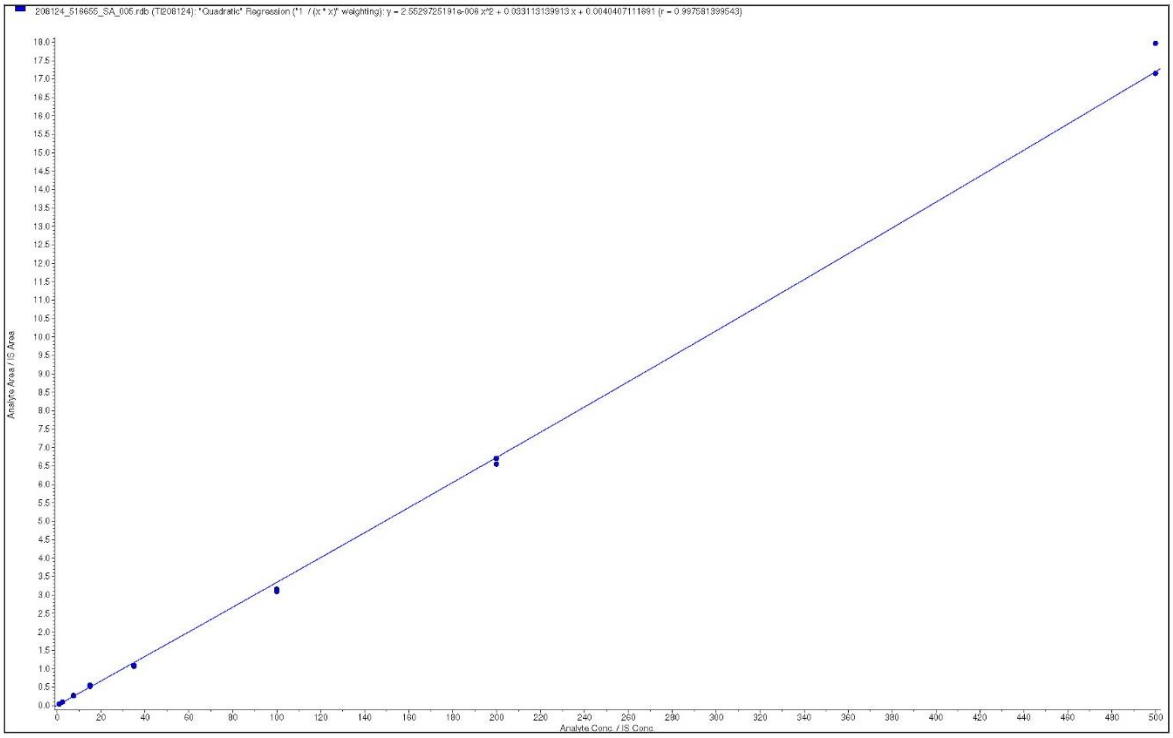

Chromatograms project: 516655
Analyst Version: 1.6.2
Results Name: 208124_516655_SA_005.rdb
Sample Name: "206366; 516655-P-8-1" Sample ID: "" File: "208124_516655_SA_0...
Peak Name: "TI208124" Mass(es): "328.900/284.800 Da"
Comment: "" Annotation: ""
Intensity, cps
Time, min
Sample Name: "206366; 516655-P-8-1" Sample ID: "" File: "208124_516655_SA_0...
Peak Name: "IS(IS)" Mass(es): "332.000/286.800 Da"
Comment: "" Annotation: ""
Intensity, cps
Time, min
Sample Name: "206367; 516655-CAR-O-1" Sample ID: "" File: "208124_516655_S...
Peak Name: "TI208124" Mass(es): "328.900/284.800 Da"
Comment: "" Annotation: ""
Intensity, cps
Time, min
Sample Name: "206367; 516655-CAR-O-1" Sample ID: "" File: "208124_516655_S...
Peak Name: "IS(IS)" Mass(es): "332.000/286.800 Da"
Comment: "" Annotation: ""
Intensity, cps
Time, min


Chromatograms project: 516655
Analyst Version: 1.6.2
Results Name: 208124_516655_SA_005.rdb
Sample Name: "206368; 516655-MATRIX" Sample ID: "" File: "208124_516655_SA...
Peak Name: "TI208124" Mass(es): "328.900/284.800 Da"
Comment: "" Annotation: ""
Sample Name: "206368; 516655-MATRIX" Sample ID: "" File: "208124_516655_SA...
Peak Name: "IS(IS)" Mass(es): "332.000/286.800 Da"
Comment: "" Annotation: ""
Sample Name: "206369; 516655-QC-L-1" Sample ID: "" File: "208124_516655_SA_...
Peak Name: "TI208124" Mass(es): "328.900/284.800 Da"
Comment: "" Annotation: ""
Sample Name: "206369; 516655-QC-L-1" Sample ID: "" File: "208124_516655_SA_...
Peak Name: "IS(IS)" Mass(es): "332.000/286.800 Da"
Comment: "" Annotation: ""
Intensity, cps
Time, min


Chromatograms project: 516655
Analyst Version: 1.6.2
Results Name: 208124_516655_SA_005.rdb
Sample Name: "206370; 516655-QC-M-1" Sample ID: "" File: "208124_516655_SA...
Peak Name: "TI208124" Mass(es): "328.900/284.800 Da"
Comment: "" Annotation: ""
Sample Name: "206370; 516655-QC-M-1" Sample ID: "" File: "208124_516655_SA...
Peak Name: "IS(IS)" Mass(es): "332.000/286.800 Da"
Comment: "" Annotation: ""
Sample Name: "206371; 516655-QC-H-1" Sample ID: "" File: "208124_516655_SA...
Peak Name: "TI208124" Mass(es): "328.900/284.800 Da"
Comment: "" Annotation: ""
Sample Name: "206371; 516655-QC-H-1" Sample ID: "" File: "208124_516655_SA...
Peak Name: "IS(IS)" Mass(es): "332.000/286.800 Da"
Comment: "" Annotation: ""
Intensity, cps
Time, min


Chromatograms project: 516655
Analyst Version: 1.6.2
Results Name: 208124_516655_SA_005.rdb
Sample Name: "206372; 516655-MATRIX" Sample ID: "" File: "208124_516655_SA...
Peak Name: "TI208124" Mass(es): "328.900/284.800 Da"
Comment: "" Annotation: ""
Sample Name: "206372; 516655-MATRIX" Sample ID: "" File: "208124_516655_SA...
Peak Name: "IS(IS)" Mass(es): "332.000/286.800 Da"
Comment: "" Annotation: ""
Sample Name: "206355; 516655:GR.A-SJ.12-PER. 1-T=-" Sample ID: "516655" Fil...
Peak Name: "TI208124" Mass(es): "328.900/284.800 Da"
Comment: "" Annotation: ""
Sample Name: "206355; 516655:GR.A-SJ.12-PER. 1-T=-" Sample ID: "516655" Fil...
Peak Name: "IS(IS)" Mass(es): "332.000/286.800 Da"
Comment: "" Annotation: ""
Intensity, cps
Time, min


Chromatograms project: 516655
Analyst Version: 1.6.2
Results Name: 208124_516655_SA_005.rdb
Sample Name: "206351; 516655:GR.A-SJ.2-PER. 1-T=-" Sample ID: "516655" File:...
Peak Name: "TI208124" Mass(es): "328.900/284.800 Da"
Comment: "" Annotation: ""
Intensity, cps
Time, min
Sample Name: "206351; 516655:GR.A-SJ.2-PER. 1-T=-" Sample ID: "516655" File:...
Peak Name: "IS(IS)" Mass(es): "332.000/286.800 Da"
Comment: "" Annotation: ""
Intensity, cps
Time, min


208124_516655_SA_005.rdb (TI208124): "Quadratic" Regression (*1 / (x * x)" weighting)): y = 2.5529725191e-006 x^2 + 0.033113139913 x + 0.0040407111691 (r = 0.997581399543)
Analyte Area / IS Area
Analyte Conc. / IS Conc.

Appendix 1
Phase Study Plan, Amendments, and Deviations

DEVIATIONS

All deviations that occurred during the study have been authorized/acknowledged by the Study Director, assessed for impact, and documented in the study records. All study plan deviations and those SOP deviations that could have impacted the quality or integrity of the study are listed below.

None of the deviations were considered to have impacted the overall integrity of the study or the interpretation of the study results and conclusions.

9.2 Internal standard

- A second batch of internal standard was also used:

Identification:	M3HFPO-DA
Appearance:	Colourless liquid (determined by Charles River Den Bosch)
Batch:	M3HFPODA0616
Purity/Composition:	50 ± 2.5 µg/mL in methanol
Chemical Purity	> 98%
Isotopic purity	≥ 99% $^{13}C(^{13}C_3)$
Storage:	in the refrigerator (2-8°C)
Stable under storage conditions until:	25 June 2019 (expiry date)
Supplier:	Wellington Laboratories Inc., Ontario, Canada
Additional information	
Test Facility analytical standard number:	AS1673/B

Evaluation: Addition of a new batch was not amended. No influence on the study results.

9.3 Study Samples

- A: - Inventory list was missing with shipments arrived on 6, 8 and 10 March.
- B: - Identification information (at least a project number) was missing on all samples of all shipments.

Evaluation:
A: Sponsor was contacted for confirmation for the number of samples to insure that the correct number of samples was received and a proposal inventory list was supplied to correct this for future deliveries.

B: The samples were marked A1 to A24 and with the shipments of 14, 20, 22 and 27 March 2017 the study number was mentioned on the inventory list. On basis of this and earlier correspondence it was sufficient to identify the samples.

charles river

FINAL BIOANALYTICAL STUDY PLAN

Test Site Study No. 516655

Clinical Trial Reference No. C30031

Determination of HFPO-DA in EDTA Human Plasma Samples

SPONSOR:
The Chemours Company
1007 Market Street
Wilmington,
DE 19899
USA

TEST SITE:
Charles River Laboratories Den Bosch BV
Hambakenwetering 7
5231 DD 's-Hertogenbosch
The Netherlands

Charles River Laboratories Den Bosch BV
Nistelrooisebaan 3
5374 RE Schaijk
The Netherlands

31 January 2017

TABLE OF CONTENTS

1. OBJECTIVE 3
2. PROPOSED STUDY SCHEDULE 3
3. GUIDELINES FOR STUDY DESIGN 3
4. REGULATORY COMPLIANCE 3
5. QUALITY ASSURANCE 3
6. SPONSOR 4
7. RESPONSIBLE PERSONNEL 4
8. TRIAL SUBJECT INTEGRITY 5
9. TEST ITEM, INTERNAL STANDARD, STUDY SAMPLES AND MATRIX 6
10. SAFETY 8
11. SAMPLE ANALYSIS 9
12. CONSTRUCTED VARIABLES 12
13. COMPUTERIZED SYSTEMS 12
14. AMENDMENTS AND DEVIATIONS 13
15. RETENTION OF RECORDS 13
16. REPORTING 13
17. REFERENCES 13
TEST SITE APPROVAL 14
SPONSOR APPROVAL 15
ATTACHMENT A 16
ATTACHMENT B 17

1. OBJECTIVE

The objective of the bioanalytical study is to analyze study samples of EDTA human plasma for the determination of HFPO-DA.

2. PROPOSED STUDY SCHEDULE

Proposed study dates are listed below. Actual applicable dates will be included in the Final Report

Experimental Start Date:	20 Feb 2017 (First date of phase-specific data collection)
Experimental Completion Date:	14 May 2017 (Last date data are collected from the phase)
Unaudited Draft Report:	09 Jun 2017

The bioanalytical study will be performed at the Schaijk location, all other supporting activities will be performed at the 's-Hertogenbosch location.

3. GUIDELINES FOR STUDY DESIGN

The design of this study was based on the study objective, the overall product development strategy for the test item, and the following study design guidelines:

- European Medicines Agency (EMA). *Guideline on Bioanalytical Method Validation.* EMEA/CHMP/EWP/192217/2009. 21 July 2011.
- FDA Guidance for Industry: *Bioanalytical Method Validation.* May 2001.
- EMA *Reflection Paper for Laboratories that Perform the Analysis or Evaluation of Clinical Trial Samples.* EMA/INS/GCP/532137/2010. 28 February 2012.
- ICH Harmonised Tripartite Guideline E6(R1). *Guideline for Good Clinical Practice.* 10 June 1996.
- UK Medicines and Healthcare Products Regulatory Agency (MHRA). *Good Clinical Practice Guidance on the Maintenance of Regulatory Compliance in Laboratories that Perform the Analysis or Evaluation of Clinical Trial Samples.* July 2009.

4. REGULATORY COMPLIANCE

The bioanalytical study will be performed in accordance with the OECD Principles of Good Laboratory Practice as accepted by Regulatory Authorities throughout the European Union, United States of America, Japan, and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

5. QUALITY ASSURANCE

The Test Site Quality Assurance Unit (QAU) will monitor the bioanalytical study to assure the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with Good Laboratory Practice regulations. The QAU will review the phase study plan, conduct inspections at intervals adequate to assure the integrity of the study, and audit the Final Phase Report to assure that it accurately describes the methods and standard operating procedures and that the reported results accurately reflect the raw data of the study.

The Test Site QAU contact is indicated below:

C.J. Mitchell, BSc.
Address as cited for Test Site
Tel: +31 73 640 6700
E-mail: christine.mitchell@crl.com

6. SPONSOR

Sponsor Representative

S. Gannon, Ph.D., DABT
Tel: +1 302 773 1376
E-mail: SHAWN.A.GANNON@chemours.com

7. RESPONSIBLE PERSONNEL

Study Director (Analytical Project Manager)

T.P. Schoutsen, MSc.
Address as cited for Test Site
Tel: +31 73 640 6700
Email: dick.schoutsen@crl.com

Management Contact

T.H.M. Noij, PhD.
Address as cited for Test Site
Tel: +31 73 640 6700
E-mail: theo.noij@crl.com

8. TRIAL SUBJECT INTEGRITY

8.1. Unscheduled Analysis as a Result of an Adverse Event

The Sponsor should clearly indicate to Charles River Den Bosch if unscheduled analysis is necessary as a result of an adverse event that could affect the subject's safety.

Agreements about adverts events (not limited to): Sponsor indicates this is not needed.

8.2. Informed Consent

The sponsor is responsible for obtaining informed consent from the clinical trial's subjects. It is anticipated that all the work contracted to Charles River Den Bosch is covered by the consent given by the trial subject. If it is not clear from the clinical protocol, upon request the sponsor will provide a written statement clarifying this issue.

The sponsor or its representative will inform Charles River Den Bosch if informed consent is withdrawn, prior to the analysis of study samples originating from the subject(s).

8.2.1. Serious Breaches

- A serious breach is likely to affect to a significant degree: the safety or physical or mental integrity of the subjects of the trial or the scientific value of the trial.
- The Sponsor and/or their representative will be notified about the possible serious breach.
- The Sponsor will be informed immediately (within 24 hours of becoming aware of it) of any relevant deviations from the analytical study plan, clinical trial protocol or from GCP principles so that they can determine if a serious breach has taken place.
- An unexpected event will also be reported immediately.
- Reporting of these incidences will be addressed in the analytical study plan but should at least be reported to the Sponsor in the first case via phone or email.

Relevant persons, phone and email addresses for emergency cases:

- T.P. Schoutsen, MSc., phone: +31 73 640 6700, email: dick.schoutsen@crl.com.

8.2.2. Other Changes regarding Samples

The Sponsor should inform Charles River Den Bosch of any changes made in the clinical trial protocol that could affect the bioanalysis this can include but is not limited to the following examples:

- changes in anti-coagulants used in blood sampling.
- changes in conditions of storage of samples prior to arrival at Charles River Den Bosch.
- changes in the sampling time schedules for trial subjects that could affect the requirements for information concerning the long-term stability data.

9. TEST ITEM, INTERNAL STANDARD, STUDY SAMPLES AND MATRIX

9.1. General

General data concerning chemicals, reagents, preparation of stock solutions, calibration standards and quality control samples (QCs) as well as the analytical conditions will be stored under project number 516652.

9.1. Test Item

Identification:	HFPO-DA
Appearance:	To be determined
Batch:	HFPODA0616
Purity/Composition:	50 ± 2.5 µg/mL in methanol
Test item storage:	in the refrigerator (2-8°C)
Stable under storage conditions until:	25 June 2019 (expiry date)
Supplier:	Wellington Laboratories Inc., Ontario, Canada
Additional information	
Test Facility test item number:	208124/A
Purity/Composition correction factor:	No correction factor required
Test item handling:	No specific handling conditions required

9.2. Internal Standard

Identification:	M3HFPO-DA
Appearance:	To be determined
Batch:	M3HFPODA0213
Purity/Composition:	50 ± 2.5 µg/mL in methanol
Chemical Purity	>98%
Isotopic purity	≥99% $^{13}C(^{13}C_3)$
Storage:	in the refrigerator (2-8°C)
Stable under storage conditions until:	21 July 2018 (expiry date)
Supplier:	Wellington Laboratories Inc., Ontario, Canada
Additional information	
Test Facility analytical standard number:	AS1673/A
Purity/Composition correction factor:	No correction factor required
Analytical standard handling:	No specific handling conditions required

9.3. Study Samples

Study samples source of human plasma (with K_2-EDTA as anticoagulant) are workers at a manufacturing plant in Dordrecht who have volunteered to participate in the study with the goal of determining if there are measureable quantities of test item in their blood. Some of these workers are in areas with potential for exposure, others are not.

Approximately 50 plasma samples are planned for analytical purposes. The actual number of study samples will be specified in the phase report.

The study samples will be shipped frozen in polypropylene tubes on dry ice to the Analytical Project Manager at Charles River Den Bosch. At least 24 hours in advance of any shipment the recipient will be notified by email as to the date and method of sample shipment. An inventory list of the samples will accompany the shipment.

Each sample will be uniquely identified by a label giving complete identification information at minimum with study number. Samples will be shipped to:

Charles River Laboratories Den Bosch BV
Attn: T.P. Schoutsen, MSc.
Hambakenwetering 7
5231 DD 's-Hertogenbosch
The Netherlands
Tel: +31 73 640 6700
Email: dick.schoutsen@crl.com

Upon arrival at Charles River Den Bosch the study samples will be stored in the ultra-low freezer (≤ -75°C) until analysis. An acknowledgement of receipt will be sent by email to the sender.

9.4. Blank Matrix

Blank human plasma with K_2-EDTA as anticoagulant will be obtained from Sera Laboratories International (Sera Lab, Burgess Hill, West Sussex, UK) and/or Charles River. Information on the gender and supplier will be specified in the report. Blank matrices will be stored in the ultra-low freezer (≤ -75°C).

9.5. Test Item and Internal Standard Characterization

The supplier will provide to the Test Facility documentation of the identity, purity, composition, and stability for the test item and internal standard. A Certificate of Analysis or equivalent documentation will be provided for inclusion in the Final Report.

9.6. Analysis of Test Item and Internal Standard

The stability of the bulk test item analytical standard and internal standard will not be determined during the course of this study.

9.7. Reserve Samples

For each batch (lot) of test item and internal standard, if practically possible a reserve sample will be collected and maintained under the appropriate storage conditions by the Test Facility and destroyed after the expiration date.

9.8. Test Item and Internal Standard Inventory and Disposition

Records of the receipt, distribution, storage, and disposition of test item and internal standard will be maintained.

10. SAFETY

The following safety instructions apply to this study:

Standard safety precautions are specified in Charles River Den Bosch procedures.

11. SAMPLE ANALYSIS

11.1. Bioanalytical Method

Quantitative analysis of the study samples will performed according to the bioanalytical method validated for the analyte in EDTA human plasma in Charles River Den Bosch project516654.

The method and any modifications to the method and/or update to the stability data will be approved in the raw data.

11.2. Analytical Runs

Each analytical run consists of at least:

- One double blank sample.
- One single blank sample.
- Calibration standards injected at the beginning and the end of the analytical run.
- Two QC samples at three concentration levels.
- Double blank samples, whenever it is appropriate.

The analytical run history will be specified in the report.

11.3. System Suitability

On each day of analysis a system suitability standard will be injected in triplicate before the start of the analytical run(s).

The retention time window, minimum ratio of peak height/peak area and resolution between the analyte or internal standard and additional components (if any) will be determined. The ratio of peak height/peak area associates peak symmetry and column efficiency.

The system suitability parameters for sample analysis are based on the following criteria:

- The retention time should be between 75% of the minimum retention time and 125% of the maximum retention time obtained with the first three accepted runs of the validation project.
- The ratio of peak height/peak area should be ≥ 75% of the lowest obtained peak height/peak area ratio found with the first three accepted runs of the validation project.
- The resolution between the analyte or internal standard and/or additional components should be > 1.5.

11.4. Carry-over

With each analytical run a double blank sample will be analyzed after each highest calibration standard to evaluate the carry-over of the analytical system.

The carry-over of the analyte in the blank sample after the highest calibration standard should be ≤ 20% of the mean response of the lowest calibration standard and ≤ 5% for the lowest calibration standard.

If necessary, injections of double blank samples will be added inside the run to avoid any contamination of a study sample by its preceding one. If the maximum carry-over is > 20% for the analyte and/or > 5% for the internal standard the effect of carry-over will be evaluated on the results of the study samples. When the contribution of the carry-over to the analyzed concentrations of the study samples is ≤ 15% it can be concluded that the carry-over has no impact on the sample results.

11.5. Calibration Curves

Calibration standards at each concentration level will be injected in duplicate. One replicate will be analyzed at the beginning and one at the end of the analytical run. A double blank sample and a single blank sample will be analyzed before the first set of calibration standards.

The response ratio of the calibration standards will be correlated with nominal concentration using regression analysis with a $1/x^2$ weighting factor. Calibration curves with back calculated accuracies in the criterion range of 80-120% for the lowest calibration standard and 85-115% for the remaining calibration standards will be accepted.

When a back calculated accuracy does not comply with the criterion range the response ratio of the calibration standard with the highest deviation will be rejected and the calibration curve will be re-evaluated. Calibration curves will be accepted when ≥ 75% of the calibration standards fulfills the acceptance criteria.

Zero is not part of the calibrated range. Blank samples will not be taken into account in the fitting procedure.

11.6. QC Samples

Study samples will be analyzed between at least two sets of quality control (QC) samples at low, middle and high level.

The analytical run will be accepted when the accuracy of the QC samples is in the criterion range of 85-115%. Results outside the criterion range may be discarded as long as per run 2/3 of the QC samples are accepted with ≥ 50% of each level.

The overall mean accuracy and coefficient of variation on the precision of the QC samples at each concentration level of all accepted runs should be within the criterion range of 85-115% and ≤ 15%. Any deviation will be justified by the Analytical Project Manager and reported.

11.7. Reanalysis of Study Samples

11.7.1. Single Reanalysis

A single reanalysis will be necessary under any of the following conditions:

- Rejection of an analytical run.
- Concentration above the highest calibration standard.
- Concentration below LLOQ for diluted sample.
- Study samples on which carry-over has an impact.
- Response of the internal standard is < 25% or >175% of the mean response obtained with the calibration standards, QC samples and single blank samples.
- Technical or analytical problem (equipment failure, incorrect addition of internal standard, sample lost during processing).

If the initial result was not valid and the reanalysis result is valid then it will be reported as the final result. When the initial result was valid the criteria used for reporting are described in ATTACHMENT B.

11.7.2. Multiple Reanalysis

Reanalysis in duplicate will be performed under the following conditions:

- Suspected result due to inconsistent pharmacokinetic behavior.
- Suspected chromatographic failure.
- Due to sample processing reasons.

The duplicate results will be used for the determination of the final result according to the flowchart in ATTACHMENT B. Duplicate reanalysis will only be performed if permitted by the available sample volume. Otherwise, single reanalysis is allowed.

11.8. Incurred Samples Reanalysis

Incurred samples reanalysis will be performed on study samples of the current study.

In a separated run on a different day, 10% of the analyzed samples with a minimum of 20 will be reanalyzed if the number of study samples of the project is ≤ 1000. The analyzed concentrations for the initial analysis and reanalysis should be ≤ 20% of their mean for ≥ 67% of the repeats.

The analyzed concentrations of the incurred samples will be reported in a separate table and won't be used for kinetic purposes. Incurred samples reanalysis only relates to the evaluation of analytical method performance. If applicable, the incurred samples can be analyzed outside the long term stability period.

If the trial concerns different distinct groups of subjects it will be represented in the selection of the incurred samples.

12. CONSTRUCTED VARIABLES

Response	Detector signal in the chromatogram expressed as peak area
Response ratio	$\frac{\text{Peak area of analyte}}{\text{Peak area of internal standard}}$
Calibration curve	Calibration measurements are subjected to least-square regression analysis
Analyzed concentration (C_A)	Analysed concentration of samples taking the dilution factor into account (if any)
Accuracy	$\frac{C_A}{C_N} \times 100$ [%] where: C_N = nominal concentration
Difference	$\frac{\text{highest value - lowest value}}{\text{mean value}} \times 100$ [%]
Resolution (R_s)[1]	$R_s = \frac{1.18\left(\lvert t_{Rb} - t_{Ra} \rvert\right)}{\left(b_{0.5a} + b_{0.5b}\right)}$ where: t_R = retention time of component a or b $b_{0.5}$ = peak width at 50% height of component a or b

13. COMPUTERIZED SYSTEMS

The following critical computerized systems may be used in the study. The actual critical computerized systems used will be specified in the Final Report.

Data for parameters not required by study plan, which are automatically generated by analytical devices used will be retained on file but not reported.

Critical Computerized Systems

System name	Description of Data Collected and/or Analyzed
Analyst	System control, data acquisition and integration
MassLynx	System control, data acquisition and integration
Labware LIMS	Laboratory information management system (LIMS)
REES Centron	Temperature, relative humidity and/or atmospheric pressure data monitoring

14. AMENDMENTS AND DEVIATIONS

Changes to the approved study plan shall be made in the form of an amendment, which will be signed and dated by the Analytical Project Manager. Every reasonable effort will be made to discuss any necessary study plan changes in advance with the Sponsor.

All study plan and SOP deviations will be documented in the study records. The Analytical Project Manager will notify the Sponsor of deviations that may result in a significant impact on the study as soon as possible.

15. RETENTION OF RECORDS

All phase-specific raw data, electronic data, documentation (e.g. relevant emails), phase study plan, and final phase report will be archived by no later than the date of final phase report issue. All materials generated by Charles River from this phase will be transferred to a Charles River archive. Five year after issue of the final phase report, the Sponsor will be contacted to determine the disposition of materials associated with the phase.

Records to be maintained will include, but will not be limited to, documentation and data for the following:

- Study plan, study plan amendments, and deviations
- Study schedule
- Study-related correspondence
- Test item receipt, identification, preparation, and analysis
- Measurements and observations

16. REPORTING

A comprehensive Draft Phase Report will be prepared following completion of the study and will be finalized following consultation with the Sponsor. The report will include all information necessary to provide a complete and accurate description of the experimental methods and results and any circumstances that may have affected the quality or integrity of the phase.

The Sponsor will receive an electronic version of the Draft Phase Report. The Final Phase Report will be provided in Adobe Acrobat PDF format (hyperlinked and searchable) along with a Microsoft Word version of the text. The PDF document will be created from native electronic files to the extent possible, including text and tables generated by the Test Site. Report components not available in native electronic files and/or original signature pages will be scanned and converted to PDF image files for incorporation. An original copy of the report with the Test Site's handwritten signatures will be retained.

Reports should be finalized within 6 months of issue of the Draft Phase Report. If the Sponsor has not provided comments to the report within 6 months of draft issue, the phase report will be finalized by the Test Site unless other arrangements are made by the Sponsor.

17. REFERENCES

1. The European Pharmacopoeia. 7th edition. 2011.

TEST SITE APPROVAL

The signature below acknowledges Test Site Management's responsibility to the study as defined by the relevant GLP regulations.

Date: 30 January 2017

E. Baltussen, PhD.

Associate Head of Chemistry

The signature below indicates that the Study Director (Analytical Project Manager) approves the study plan.

Date: 31 January 2017

T.P. Schoutsen, MSc.

Study Director

SPONSOR APPROVAL

The signature of the Sponsor Representative below indicates approval of this study plan.

Date: 23 Oct 2017

S. Gannon, Ph.D., DABT

Sponsor Representative

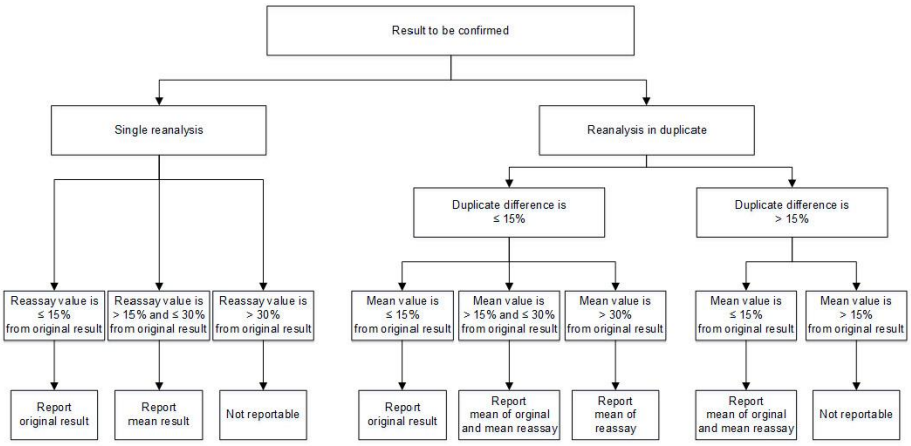
ATTACHMENT A

Distribution List

Version	Recipient
Original	Study Director
1 Copy	Sponsor
1 Copy	QAU/Management

ATTACHMENT B

Reanalysis Flowchart



charles river

STUDY PLAN AMENDMENT NUMBER: 01

Study Title	Determination of HFPO-DA in EDTA Human Plasma Samples
Sponsor	The Chemours Company 1007 Market Street Wilmington, DE 19899 USA
Sponsor Representative	S. Gannon, Ph.D., DABT Tel: +1 302 773 1376 E-mail: SHAWN.A.GANNON@chemours.com
Test item	208124/A
Test Facility Project	516655
Sponsor Reference No.	C30031

AMENDMENT DESCRIPTION

1. Page 6, paragraph 9. TEST ITEM, INTERNAL STANDARD, STUDY SAMPLES AND MATRIX
   Paragraph 9.1 Test Item should be:
   9.2 Test Item, all the other headings are updated to subsequently to 9.3, 9.4 up to 9.9, respectively.

2. Page 6, paragraph 9.2. Test Item:

   Appearance: **Colourless liquid (determined by Charles River Den Bosch)**

3. Page 6, paragraph 9.3. Internal Standard:

   Appearance: **Colourless liquid (determined by Charles River Den Bosch)**

STUDY PLAN AMENDMENT NUMBER: 01 (continued)

REASONS FOR AMENDMENT

1. Update of the headings, Original headings were not correct.

2+3 The data has been updated, according to information determined by Charles River Den Bosch, dated 01 February 2017.

APPROVAL
Study director

T.P. Schoutsen MSc.

Date: 08 February 2017.

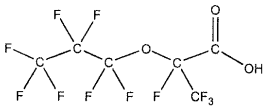
Appendix 2
Test Item Characterization

WELLINGTON LABORATORIES

CERTIFICATE OF ANALYSIS
DOCUMENTATION

PRODUCT CODE: HFPO-DA **LOT NUMBER:** HFPODA0616
COMPOUND: 2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-propanoic acid

STRUCTURE: **CAS #:** 13252-13-6



MOLECULAR FORMULA: $C_6HF_{11}O_3$ **MOLECULAR WEIGHT:** 330.05
CONCENTRATION: 50 ± 2.5 µg/ml **SOLVENT(S):** Methanol
CHEMICAL PURITY: >98%
LAST TESTED: (mm/dd/yyyy) 06/25/2016
EXPIRY DATE: (mm/dd/yyyy) 06/25/2019
RECOMMENDED STORAGE: Store ampoule in a cool, dark place

DOCUMENTATION/ DATA ATTACHED:

Figure 1: LC/MS Data (TIC and Mass Spectrum)
Figure 2: LC/MS/MS Data (Selected MRM Transitions)

ADDITIONAL INFORMATION:

- See page 2 for further details.
- Product is commercially known as GenX.

FOR LABORATORY USE ONLY: NOT FOR HUMAN OR DRUG USE

Certified By: B.G. Chittim **Date:** 07/14/2016 (mm/dd/yyyy)

Wellington Laboratories Inc., 345 Southgate Dr. Guelph ON N1G 3M5 CANADA
519-822-2436 • Fax: 519-822-2849 • info@well-labs.com

Appendix 3
Internal Standard Characterization

AS1673/A

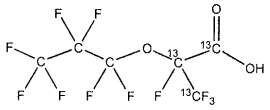
WELLINGTON LABORATORIES

CERTIFICATE OF ANALYSIS
DOCUMENTATION

PRODUCT CODE: M3HFPO-DA **LOT NUMBER:** M3HFPODA0213

COMPOUND: 2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-$^{13}C_3$-propanoic acid

STRUCTURE: **CAS #:** Not available



MOLECULAR FORMULA: $^{13}C_3{}^{12}C_3HF_{11}O_3$ **MOLECULAR WEIGHT:** 333.03

CONCENTRATION: 50 ± 2.5 µg/ml **SOLVENT(S):** Methanol

CHEMICAL PURITY: >98% **ISOTOPIC PURITY:** ≥99% ^{13}C ($^{13}C_3$)

LAST TESTED: (mm/dd/yyyy) 07/21/2015

EXPIRY DATE: (mm/dd/yyyy) 07/21/2018

RECOMMENDED STORAGE: Store ampoule in a cool, dark place

DOCUMENTATION/ DATA ATTACHED:

Figure 1: LC/MS Data (TIC and Mass Spectrum)
Figure 2: LC/MS/MS Data (Selected MRM Transitions)

ADDITIONAL INFORMATION:

- See page 2 for further details.
- Contains ~ 1.5% of two constitutional isomers.

FOR LABORATORY USE ONLY: NOT FOR HUMAN OR DRUG USE

Certified By: B.G. Chittim **Date:** 07/23/2015 (mm/dd/yyyy)

Wellington Laboratories Inc., 345 Southgate Dr. Guelph ON N1G 3M5 CANADA
519-822-2436 • Fax: 519-822-2849 • info@well-labs.com

AS1673/B

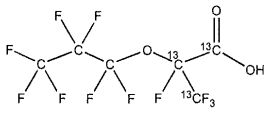
WELLINGTON LABORATORIES

CERTIFICATE OF ANALYSIS
DOCUMENTATION

PRODUCT CODE: M3HFPO-DA **LOT NUMBER:** M3HFPODA0616

COMPOUND: 2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-$^{13}C_3$-propanoic acid

STRUCTURE: **CAS #:** Not available



MOLECULAR FORMULA:	$^{13}C_3{}^{12}C_3HF_{11}O_3$	**MOLECULAR WEIGHT:**	333.03
CONCENTRATION:	50 ± 2.5 µg/ml	**SOLVENT(S):**	Methanol
CHEMICAL PURITY:	>98%	**ISOTOPIC PURITY:**	≥99% ^{13}C ($^{13}C_3$)
LAST TESTED: (mm/dd/yyyy)	06/25/2016		
EXPIRY DATE: (mm/dd/yyyy)	06/25/2019		
RECOMMENDED STORAGE:	Store ampoule in a cool, dark place		

DOCUMENTATION/ DATA ATTACHED:

Figure 1: LC/MS Data (TIC and Mass Spectrum)
Figure 2: LC/MS/MS Data (Selected MRM Transitions)

ADDITIONAL INFORMATION:

- See page 2 for further details.
- Contains ~ 1.5% of two constitutional isomers.
- Product is commercially known as GenX.

FOR LABORATORY USE ONLY: NOT FOR HUMAN OR DRUG USE

Certified By: B.G. Chittim **Date:** 06/29/2016 (mm/dd/yyyy)

Wellington Laboratories Inc., 345 Southgate Dr. Guelph ON N1G 3M5 CANADA
519-822-2436 • Fax: 519-822-2849 • info@well-labs.com

Appendix 4
Bioanalytical Method

charles river

Charles River Den Bosch
STF CHE/K/001A/13

STF modified for
Bioanalytical base project 516652 Test item 208124 Version 05

BIOANALYTICAL PROCEDURE

BIOANALYTICAL METHOD

Human plasma

Linked to method validation in Charles River Den Bosch project 516654

Method approved by:
T.P. Schoutsen, MSc.
Study director

Date: 07 Jan 2017

Method released by:
E. Baltussen, PhD.
Head of Chemistry

Date: 08 June 2017

charles river

Charles River Den Bosch
STF CHE/K/001A/13

STF modified for
Bioanalytical base project 516652 Test item 208124 Version 05

ANALYTICAL CONDITIONS

Instrument	Acquity UPLC system (Waters, Milford, MA, USA)
Detector	API 4000 mass spectrometer (AB Sciex, Toronto, Canada)
Column	Acquity UPLC BEH C18, 50 × 2.1 mm i.d., dp = 1.7 µm (Waters)
Inline filter/guard column	Inline filter ASSY frit, 2.1 mm id., 0.2 µm (Waters)
Column oven temperature	50°C ± 1°C
Autosampler temperature	4°C
Injection volume	5 µL
Injection loop	10 µL
Needle	30 µL stainless steel needle
Sample syringe	100 µL
Eluent A	10/85/5 (v/v/v) acetonitrile/water/50 mM ammonium formate in water
Eluent B	90/5/5 (v/v/v) acetonitrile/water/50 mM ammonium formate in water

Gradient

Time [minutes]	Eluent A [%]	Eluent B [%]
0.00	90.0	10.0
0.30	90.0	10.0
1.50	10.0	90.0
1.60	90.0	10.0
2.00	90.0	10.0

Flow	0.600 mL/min
Run time	2 minutes
Strong wash	25/25/25/25/0.1 (v/v/v/v/v) 2-propanol/acetonitrile/methanol/ water/formic acid
Weak wash	10/90 (v/v) acetonitrile/water

MS/MS conditions

Ionisation source	ESI^-
Source temperature	450°C
Ionspray voltage	-4000V
Source parameters	x = 5 y = 2

Acquisition

Compound	MRM monitored	Declustering potential (V)	Collision energy (eV)	Collision cell exit potential (V)	Dwell time (ms)
Analyte	*m/z* 328.9 -> *m/z* 284.8	-30	-8	-7	100
Internal standard	*m/z* 332.0 -> *m/z* 286.8	-35	-10	-7	100

Switching valve program

Time [minutes]	Position
0.0	To waste
0.7	To MS

Approval
Study director 05 Jan 2017
Competent person -

charles river

Charles River Den Bosch
STF CHE/K/001A/13

STF modified for
Bioanalytical base project 516652 Test item 208124 Version 05

System control, data acquisition and data processing will be performed using the following program:
- Analyst version 1.6.2 (AB Sciex, Toronto, Canada).

STOCK AND STANDARD SOLUTIONS

The standard preparation scheme for stock and standard solutions is a suggested approach. Appropriate modifications to reach the targeted nominal concentrations for standard solutions and QC samples are acceptable.
Prepare stock solutions of the analyte and internal standard in glass vessels. Prepare all other solutions, standards and samples in polypropylene tubes.
No correction factor is required for the analyte and internal standard.

Stock solutions

Analyte stock solutions
The certified test item solution of 50.0 µg/mL TI208214 in methanol was divided into portions and stored. Aliquot stock solutions in glass vials with insert and store in the ultra-low freezer (≤ -75°C).

Internal standard stock solutions
The certified test item solution of 50.0 µg/mL AS1673 in methanol was divided into portions and stored. Aliquot stock solutions in glass vial with insert and store in the ultra-low freezer (≤ -75°C).

Working and spiking solutions

Analyte working and spiking solutions
Dilute the stock solutions of the analyte in Methanol to obtain working and spiking solutions. A preparation scheme for the working and spiking solutions is given in Table 1.

Prepare working and spiking solutions on the day of use. If applicable, aliquot spiking solutions in polypropylene tubes and store in the ultra-low freezer (≤ -75°C).

Table 1 Preparation scheme of analyte working and spiking solutions

Code	Applied solution	Volume applied solution [µL]	Volume applied solvent [µL]	Target concentration [ng/mL]	
W1	W3	120	780	20.0	
W2	W4	150	750	50.0	
W3	W5	180	660	150	
W4	W6	150	850	300	
W5	W7	175	825	700	
W6	W8	200	800	2000	
W7	Stock I	32.0	368	4000	
W8	Stock I	70.0	280	10000	
SP-LLOQ	SP-M	20.0	480	20.0	
SP-L	SP-M	60.0	440	60.0	
SP-M	SP-H	40.0	560	500	
SP-H	Stock I	75.0	425	7500	
SP-Dil	Stock I	150	50	37500	
SP-Rec-L	SP-L	25.0	175	7.50	
SP-Rec-M	SP-M	25.0	175	62.5	
SP-Rec-H	SP-H	25.0	175	937.5	
SP-Matrix-L	SP-L	60.0	411	7.50	Analyte
	IS-Dil	9.00		93.8	IS
SP-Matrix-H	SP-H	60.0	411	938	Analyte
	IS-Dil	9.00		93.8	IS

Approval
Study director sh 07 Jan 2017
Competent person –

charles river

Charles River Den Bosch
STF CHE/K/001A/13

STF modified for
Bioanalytical base project 516652 Test item 208124 Version 05

Internal standard working solutions
Dilute stock solution of the internal standard according to the preparation scheme in Table 2 to obtain internal standard working solutions.

Aliquot internal standard solutions in polypropylene tubes and store in the ultra-low freezer (≤ -75°C). Prepare IS_work freshly on the day of use.

Table 2 Preparation scheme of internal standard working solutions

Code	Applied solution	Volume applied solution [µL]	Applied solvent	Volume applied solvent [mL]	Target concentration [ng/mL]
IS_Dil	IS-Stock	200	Methanol	1.80	5000
IS_work	IS_Dil	50.0	Acetonitrile	19.95	12.5

Calibration standards

Apply working solutions of the analyte to prepare matrix-matched calibration standards. Add blank matrix to an aliquot of the appropriate working solution and homogenize. A preparation scheme for the calibration standards is given in Table 3.

Prepare calibration standards freshly on the day of use.

Table 3 Preparation scheme of calibration standards

Code	Applied solution	Volume applied solution [µL]	Volume applied blank matrix [µL]	Target concentration [ng/mL]
C1	W1	10.0	190	1.00
C2	W2	10.0	190	2.50
C3	W3	10.0	190	7.50
C4	W4	10.0	190	15.0
C5	W5	10.0	190	35.0
C6	W6	10.0	190	100
C7	W7	10.0	190	200
C8	W8	10.0	190	500

Quality control (QC) samples

Apply spiking solutions of the analyte to prepare QC samples. Spike the appropriate spiking solution to blank matrix and homogenize. A preparation scheme for the QC samples is given in Table 4.

Aliquot QC samples in polypropylene tubes and store in the ultra-low freezer (≤ -75°C).

Table 4 Preparation scheme of QC samples

Code	Applied solution	Volume applied solution [µL]	Volume applied blank matrix [µL]	Target concentration [ng/mL]
QC-LLOQ	SP-LLOQ	50.0	950	1.00
QC-L	SP-L	50.0	950	3.00
QC-M	SP-M	50.0	950	25.0
QC-H	SP-H	50.0	950	375
QC-Dil	SP-Dil	50.0	950	1875

Approval
Study director Sch 07 Jun 2017
Competent person -

charles river

Charles River Den Bosch
STF CHE/K/001A/13

STF modified for
Bioanalytical base project 516652 Test item 208124 Version 05

System suitability standards

Prepare system suitability standards in 10/90 (v/v) Acetonitrile/Water. A preparation scheme for the system suitability standards is given in Table 5.

Aliquot system suitability standards in polypropylene vials and store in the ultra-low freezer (≤ -75°C).

Table 5 Preparation scheme of system suitability standards

Code	Applied solution	Volume applied solution [µL]	Volume applied solvent [mL]	Target concentration [ng/mL]	Component
SST	W2 IS-Dil	32.0 12.5	19.95	0.0800 3.13	Analyte IS

Recovery and matrix effect samples

Prepare recovery and matrix effect samples for validation purpose only.

Recovery samples
Process per concentration level in triplicate blank matrix with the addition of internal standard. Spike the analyte to the processed samples according to the preparation scheme in Table 6.

Table 6 Preparation scheme of recovery samples

Code	Volume processed blank [µL]	Applied solution	Volume applied solution [µL]	Target concentration[1] [ng/mL]
Rec-L	300	SP-Rec-L	10.0	0.250
Rec-M	300	SP-Rec-M	10.0	2.08
Rec-H	300	SP-Rec-H	10.0	31.3

[1] No correction is required if the spiking volume is ≤ 5% (v/v) of the volume of the processed blank.

Matrix effect samples
Process per concentration level in duplicate blank matrix with the addition of 75 µL Acetonitrile. Spike the analyte and internal standard to the processed samples according to the preparation scheme in Table 7.

As reference, spike in triplicate the analyte and internal standard to the same volume of 10/90 (v/v) Acetonitrile/Water.

Table 7 Preparation scheme of matrix effect samples

Code	Volume processed blank [µL]	Applied solution	Volume applied solution [µL]	Target concentration[1] [ng/mL]	Component
Matrix-L	300	SP-Matrix-L	10.0	0.250 3.13	Analyte IS
Matrix-H	300	SP-Matrix-H	10.0	31.3 3.13	Analyte IS

[1] No correction is required if the spiking volume is ≤ 5% (v/v) of the volume of the processed blank.

Approval
Study director 5 of Jan 2017
Competent person –

charles river

Charles River Den Bosch
STF CHE/K/001A/13

STF modified for
Bioanalytical base project 516652 Test item 208124 Version 05

Stability samples

Prepare stability samples for validation purpose only.

Stock solutions
Analyte stock solutions
Dilute stock solutions of the analyte according to the preparation scheme in Table 8 to obtain solutions of the analyte at a target concentration level within the calibrated range.

Table 8 Preparation scheme of analyte stock stability solutions

Code	Applied solution	Volume applied solution [µL]	Applied solvent	Volume applied solvent [µL]	Target concentration [ng/mL]
Stock-Dil I	Stock	10.0	Methanol	990	500
Stock-Dil II	Stock-Dil I	30.0	10/90 (v/v) Acetonitrile/Water	970	15.0

QC samples
Process stored QC samples similarly to the regular QC samples.

Whole blood
Aliquots of fresh blood will be placed at 37°C for at least 30 minutes. The analyte will be spiked at QC-low and high corresponding plasma levels according to the preparation scheme in Table 9 and gently mixed for 5 minutes at 37°C.

Place the aliquots at room temperature conditions. At t=0 and stability time points, centrifuge the aliquots at about 1500 g and 4°C for 5 minutes to obtain plasma phases. If applicable, store the plasma samples in polypropylene tubes in the ultra-low freezer (≤ -75°C) until analysis.

Table 9 Preparation scheme of blood stability samples

Code	Applied solution	Volume applied solution [µL]	Volume applied blank blood [µL]	Target concentration[1] [ng/mL]
WB-L	SP-L	25.0	975	3.00
WB-H	SP-H	25.0	975	375

[1] Based on plasma, taking into account that blood contains about 50% of plasma.

Blank matrix samples

Process blank matrix with the addition of internal standard to obtain single blank samples.

Process blank matrix with the addition of 75 µL Acetonitrile to obtain double blank samples.

If necessary, double blank matrix will be injected within sets of samples to prevent analytical results on artifacts from previous injections.

Approval
Study director 07 Jan 2017
Competent person –

charles river

Charles River Den Bosch
STF CHE/K/001A/13

STF modified for
Bioanalytical base project 516652 Test item 208124 Version 05

SAMPLE PROCESSING PROCEDURE

Thaw QC samples and study samples at room temperature conditions and homogenize.
If applicable, dilute QC and/or study samples with blank matrix and homogenize

Return study samples to the ultra-low freezer (≤ -75°C) after processing.

Select the sample preparation method using the following conditions:

- Add 75 µL of IS-work or acetonitrile into a 96-well Isolute PPT+ (Biotage) equipped with a 1mL 96-well collection plate
- Add 25 µL of calibration standard, QC, study sample or blank matrix
- Homogenize
- Elute the mixture into the collection plate by positive pressure at approximately 2 bars for about 30 seconds followed by 4 bars for about 2 seconds
- Add 200 µL of water on the collection plate
- Homogenize
- Subject to LC-MS analysis

The sample processing procedure dilutes the analyte and internal standard 12 and 4 times, respectively.

Approval
Study director 07 Jun 2017
Competent person –

charles river

Charles River Den Bosch
STF CHE/K/001A/13

STF modified for
Bioanalytical base project 516652 Test item 208124 Version 05

SYSTEM SUITABILITY TEST CRITERIA

Parameter	Compound	Data
Retention time	Analyte Internal standard	0.75 – 1.26 minutes 0.75 – 1.26 minutes
Ratio peak height / peak area	Analyte Internal standard	≥ 0.311 ≥ 0.450

NA = not applicable
ND = none determined

STABILITY

Parameter	Data	
Number of samples per run with duplicate QC samples	89	samples
Dilution integrity of samples	5	times
Stability of stock solutions of analyte at room temperature	6	hours
Stability of stock solutions of analyte in the ultra-low freezer (≤ -75°C)	25	days
Stability of W-solutions in the ultra-low freezer (≤ -75°C)	8	days
Freeze/thaw stability	3	cycles
Short term matrix stability at room temperature conditions	Rejected after 5 hours[1]	
Stability in processed samples in the refrigerator/autosampler	Rejected after 49 hours[2]	
Long term matrix stability in the ultra-low freezer (≤ -75°C)	29	days
Stability in whole blood at room temperature conditions	120	minutes
Confirmation of liquid handling system for sample processing	No	

[1] the results of the matrix stability was evaluated that it did not affect the reliability of the results as long as the QCs in a run were accepted.
[2] Samples can be stored and reanalyzed when the whole analytical run is re-injected, instead of re-injection with freshly prepared calibration curve.

VERSION HISTORY

Version 00

- First version of the bioanalytical method.

Version 01

- Bioanalytical procedure adjusted to use of 1 stock.

Version 02

- Changed container for stocks
- Changed Ostro for PPT+

Version 03

- Changed solvent for stocks to Methanol
- Changed storage temperature from freezer ≤-15°C to ultra-low freezer ≤-75°C.

Version 04

- Filled in information SST criteria and stability table.

Version 05

- Added additional data to the stability table.

Approval
Study director Sk 07 Jan 2017
Competent person –