PDF #3

27 May 2016

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Final Letter Report
Ref: WIL-361514

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Presented is the final letter report for the study entitled "*In Vitro* Stability of Cyclic (PMT9C151) and Linear (PMT9L152) Peptide in Simulated Gastric and Intestinal Fluids".

Quality control analysis results are presented in Table 1 through Table 4; cyclic (PMT9C151) peptide stability results are presented in Table 5 through Table 7; linear (PMT9L152) peptide stability is presented in Table 8 through Table 10. Representative chromatograms are presented in Figure 1 through Figure 7. The study protocol is presented in Appendix A, and test material data are presented in Appendix B.

INTRODUCTION

The objective of this study was to determine the *in vitro* stability of cyclic and linear peptides in simulated gastric and intestinal fluids. This model is used to determine the potential for test article degradation *in vivo* prior to absorption. Gastric-intestinal stability experiments were conducted on 14-Mar-2016 and analysis of samples was performed from 15-Mar-2016 through 17-Mar-2016.

METHODOLOGY

Simulated gastric and intestinal fluids (SGF and SIF, respectively) were prepared according to the instructions in the United States Pharmacopeia (USP 36). The stability of cyclic (PMT9C151) and linear (PMT9L152) peptides were evaluated at a concentration of 500 µg/mL in Milli-Q water, SGF (with enzymes), and SIF (with enzymes). Control incubations in Milli-Q water were run concurrently.

Test Article Incubations:

- Incubations were performed in capped glass vials in a shaking water bath at approximately 37°C.
- Duplicate vials (0.5-mL each) of test article in water or simulated fluids were prepared for each time point.
- At 0, 0.5, 2, 4, and 8 (cyclic) and 0, 2, and 8 (linear) hours, water reactions were divided into approximately equal aliquots, flash-frozen, and stored at approximately -70°C until analysis.
- At 0, 0.5, 1, 2, and 4 (cyclic) and 0, 2, and 4 (linear) hours, SGF reactions (with enzymes) were divided into approximately equal aliquots, flash-frozen, and stored at approximately -70°C until analysis.
- At 0, 2, 4, 6, and 8 (cyclic) and 0, 2, 8 (linear) hours, SIF reactions (with enzymes) were divided into approximately equal aliquots, flash-frozen, and stored at approximately -70°C until analysis.
- Analysis of the samples was conducted using an HPLC-UV method.

Laboratory Method and Qualification:

The analytical laboratory method and qualification results can be found in Appendix C.

RESULTS AND DISCUSSION

Cyclic Peptide stability:

Milli-Q water: Cyclic peptide was stable through 8 hours following incubation with Milli-Q water with percent of time zero ranging from 95% to 106%.

SGF: Cyclic peptide was stable through 4 hours following incubation with SGF, with percent of time zero ranging from 94.7% to 101%.

SIF: Following incubation with SIF, there was a trend for decrease in stability with increase in incubation time. The percent of time zero ranged from 60.1% to 87.5%, with lowest stability observed following 8 hours of incubation.

Linear Peptide Stability:

Milli-Q water: There was a trend for decrease in stability with increase in incubation time for linear peptide. The percent of time zero for linear peptide was 75% and 19.8% following 2 and 8 hours of incubations, respectively.

SGF: Following incubation with SGF, there was no peak observed for linear peptide at 2 and 4 hours of incubation, indicating lack of stability.

SIF: Following incubation with SIF, there was no peak observed for linear peptide in any of the samples, including at the zero hour. Slight conversion from linear to cyclic peptide was observed only in 1 of the samples tested (linear peptide, incubation with Milli-Q water for 8 hours – Figure 5).

CONCLUSIONS

Cyclic peptide was stable through 8 hours following incubation with water, 4 hours following incubation with SGF; however, following incubation with SIF, there was a trend for decrease in stability with increase in time through 8 hours of incubation time.

Linear peptide was not stable following incubations with water, SGF, and SIF. In water, linear peptide showed a decrease in stability through 8 hours tested. In SGF, there were no peaks observed for linear peptide at 2 and 4 hours of incubation. In SIF, there were no peaks observed for linear peptide in any of the samples including at the zero hour.

Sincerely,

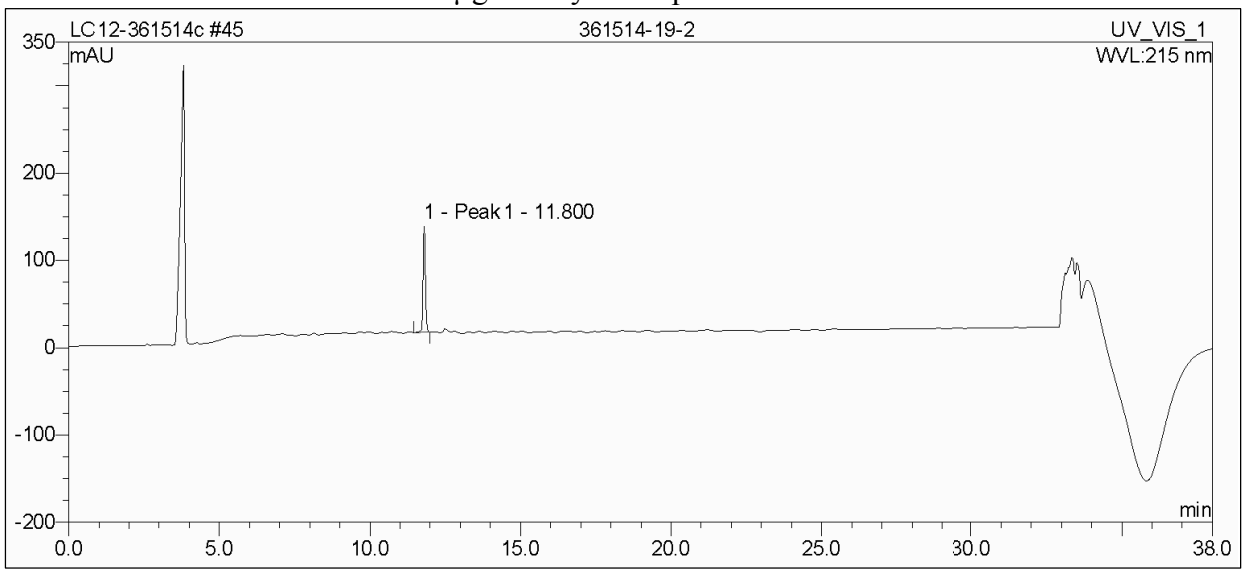
Prathap N. Shastri, PhD
Study Director
Research Scientist, ADME/DMPK

Enclosure

FIGURES

Figure 1. Representative Chromatograms for Cyclic and Linear Peptide Standard Samples at 50.0 µg/mL Concentrations

50.0 µg/mL Cyclic Peptide Standard



50.0 µg/mL Linear Peptide Standard

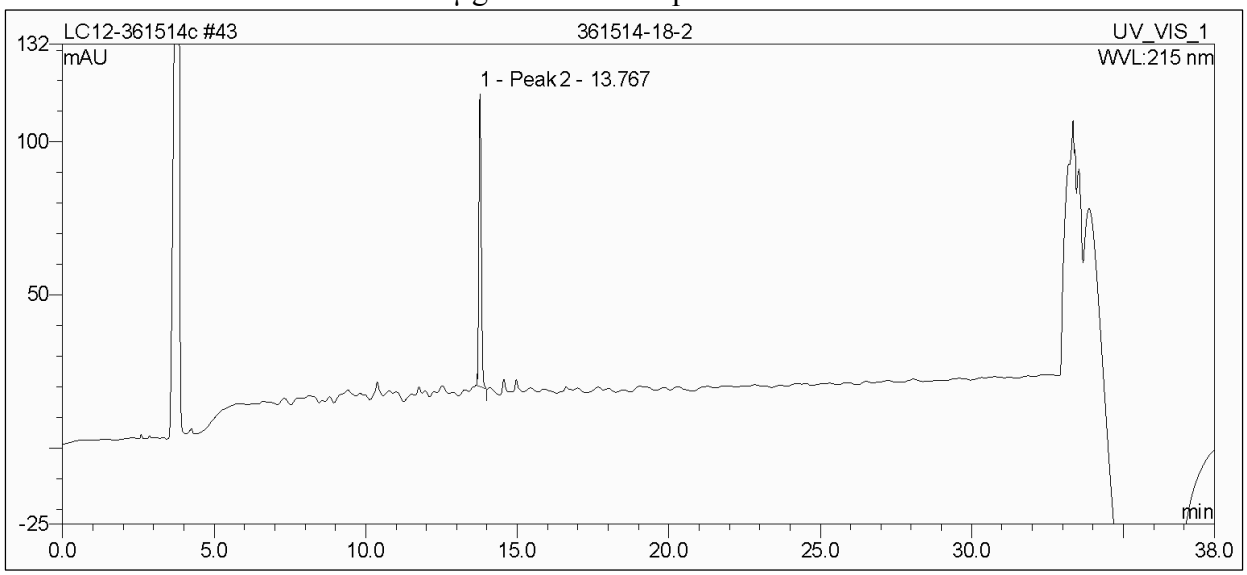
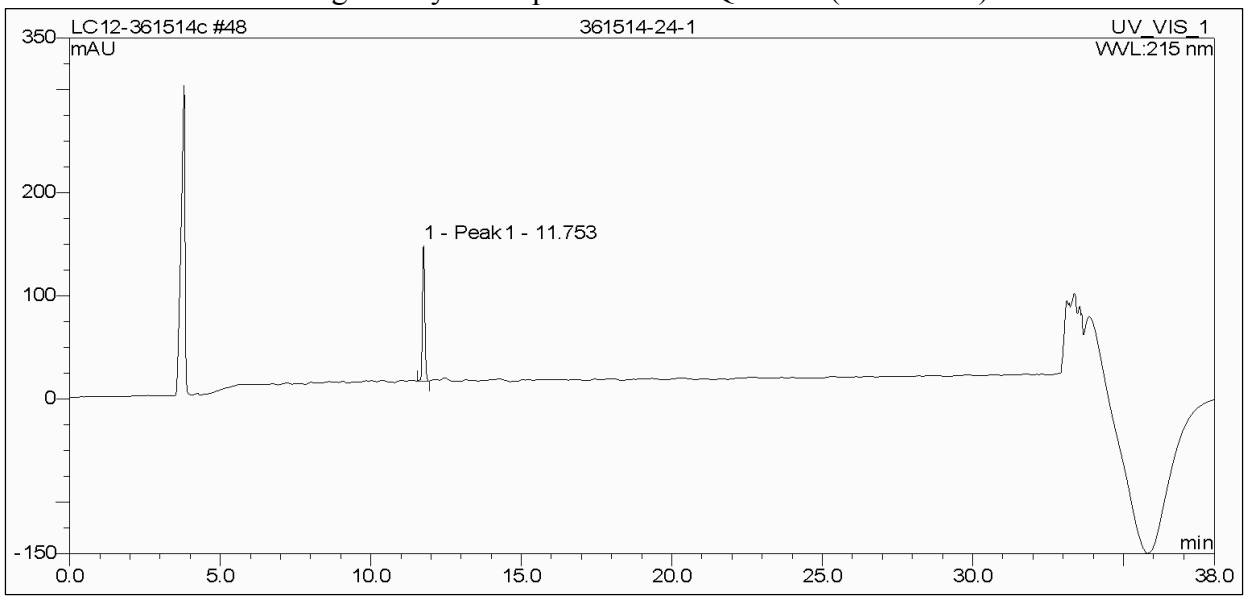


Figure 2. Representative Chromatograms for Cyclic Peptide Samples Obtained during GI Stability Experiments in Milli-Q Water

0.5 mg/mL Cyclic Peptide in Milli-Q Water (t = 0 hours)



0.5 mg/mL Cyclic Peptide in Milli-Q Water (t = 8 hours)

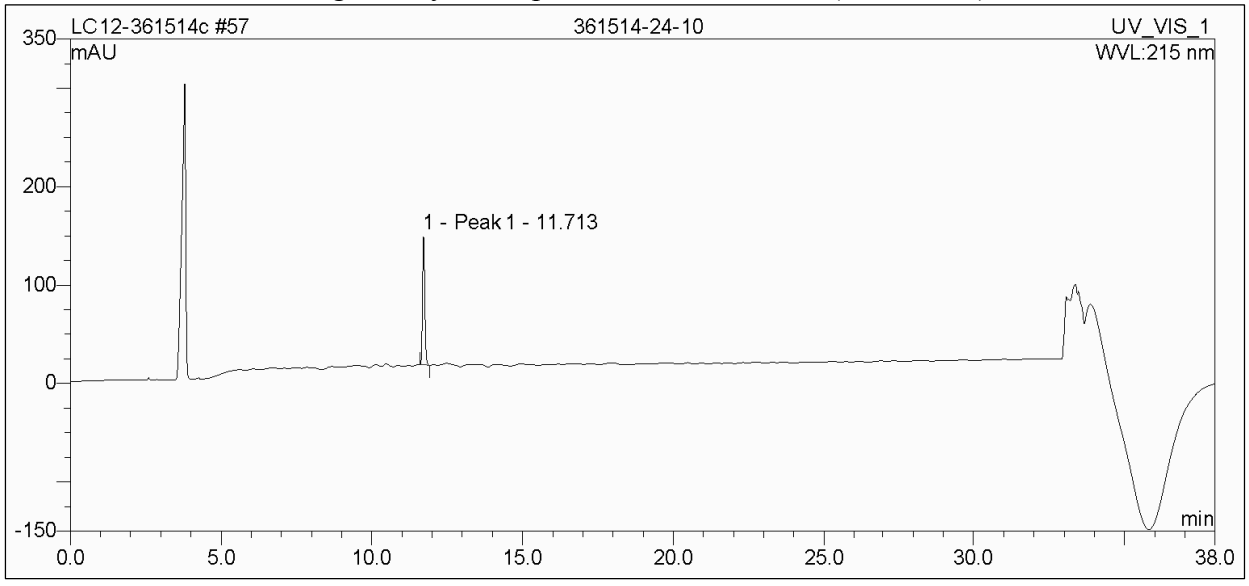
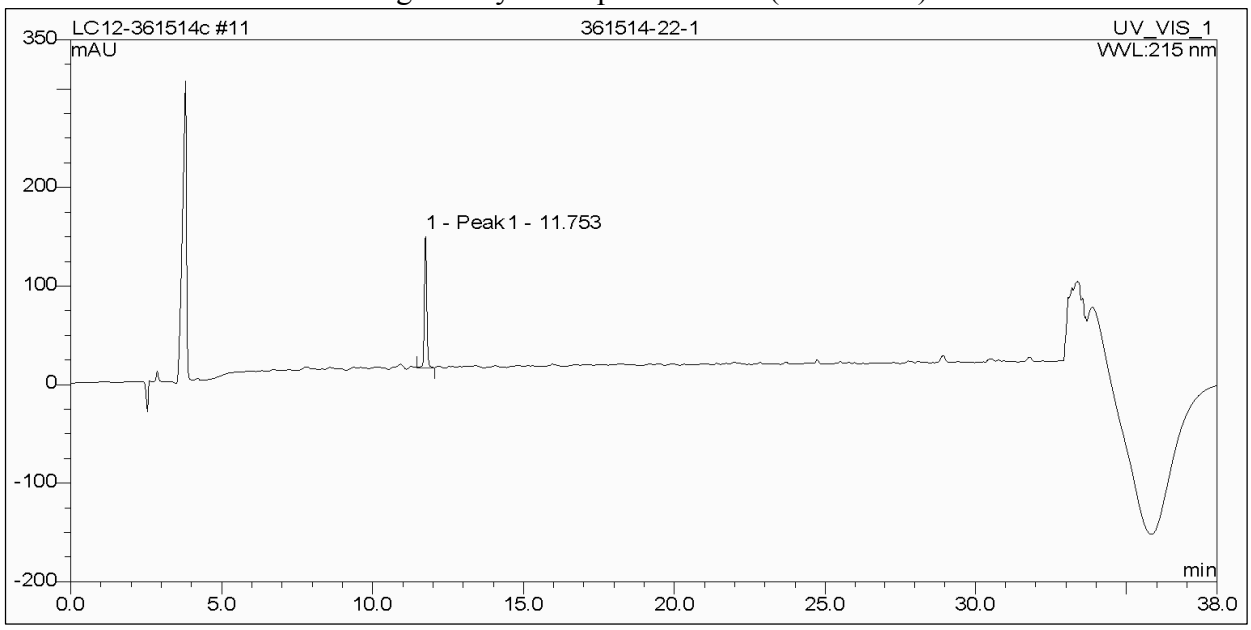


Figure 3. Representative Chromatograms for Cyclic Peptide Samples Obtained during GI Stability Experiments in SGF

0.5 mg/mL Cyclic Peptide in SGF (t = 0 hours)



0.5 mg/mL Cyclic Peptide in SGF (t = 4 hours)

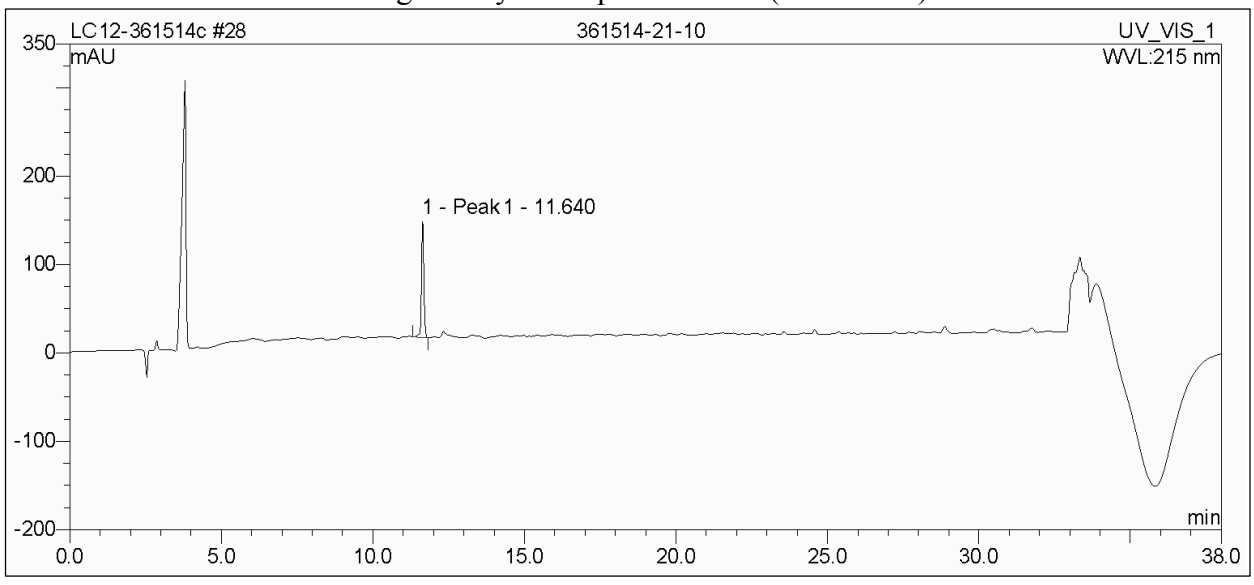
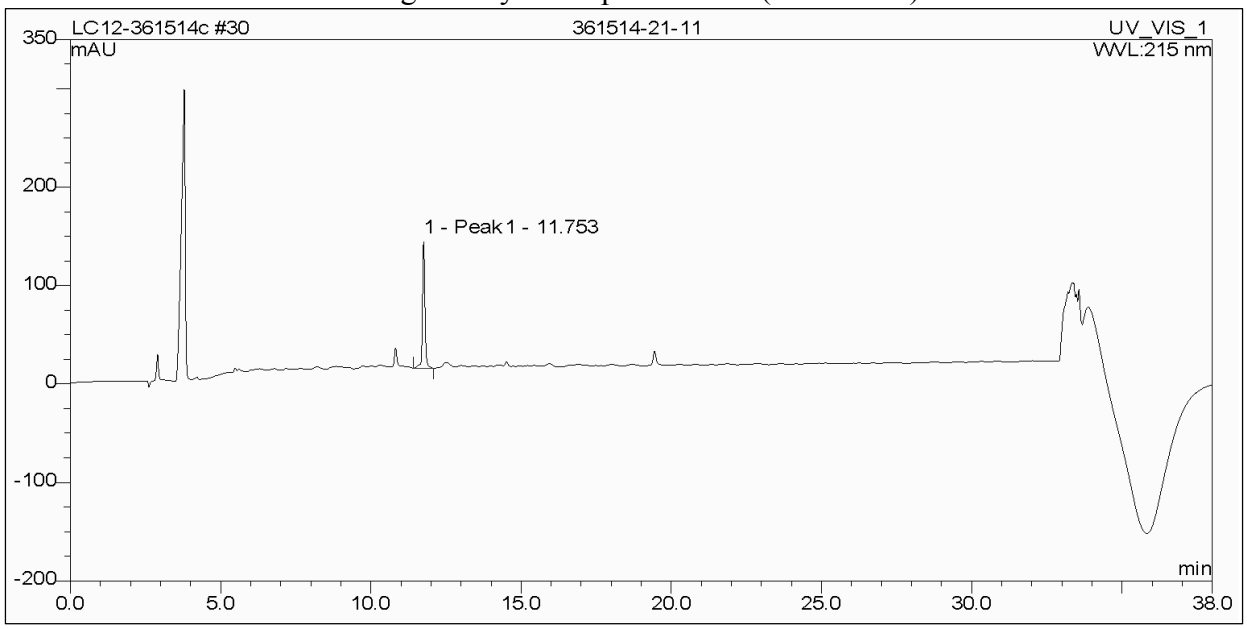


Figure 4. Representative Chromatograms for Cyclic Peptide Samples Obtained during GI Stability Experiments in SIF

0.5 mg/mL Cyclic Peptide in SIF (t = 0 hours)



0.5 mg/mL Cyclic Peptide in SIF (t = 8 hours)

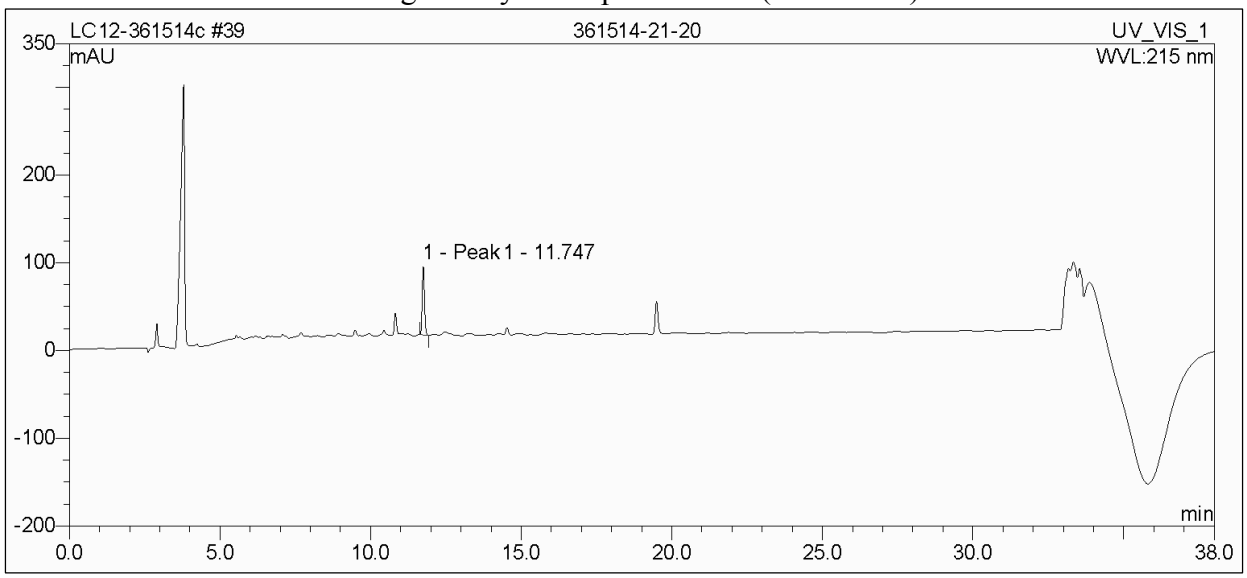
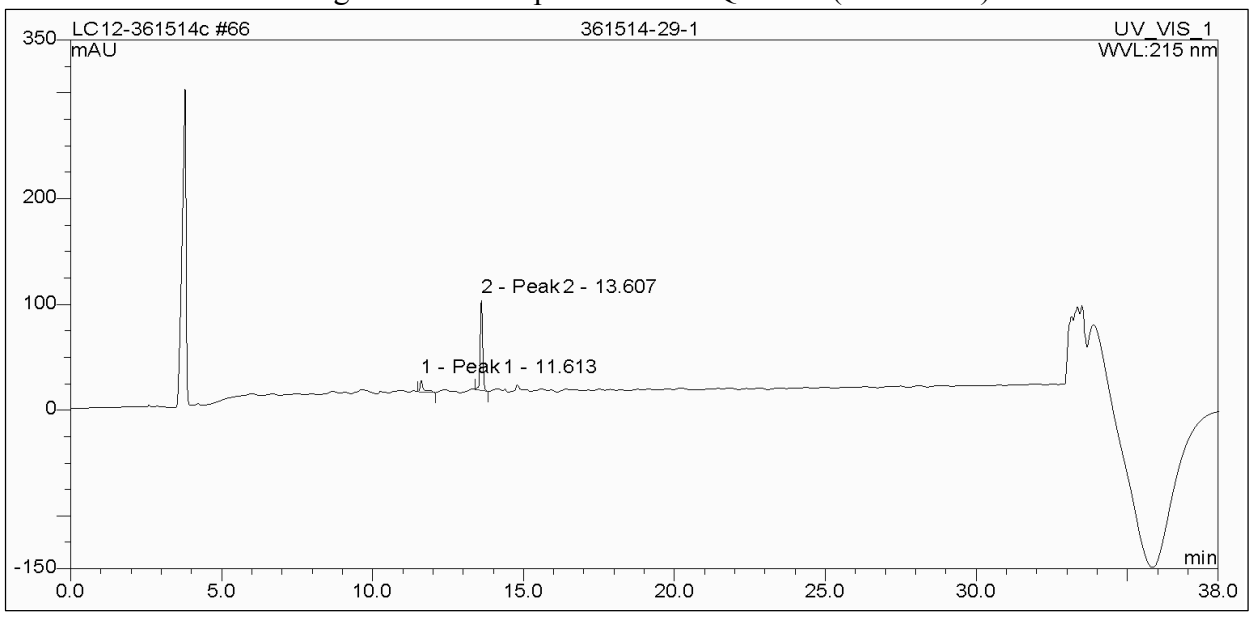


Figure 5. Representative Chromatograms for Linear Peptide Samples Obtained during GI Stability Experiments in Milli-Q Water

0.5 mg/mL Linear Peptide in Milli-Q Water (t = 0 hours)



0.5 mg/mL Linear Peptide in Milli-Q Water (t = 8 hours)

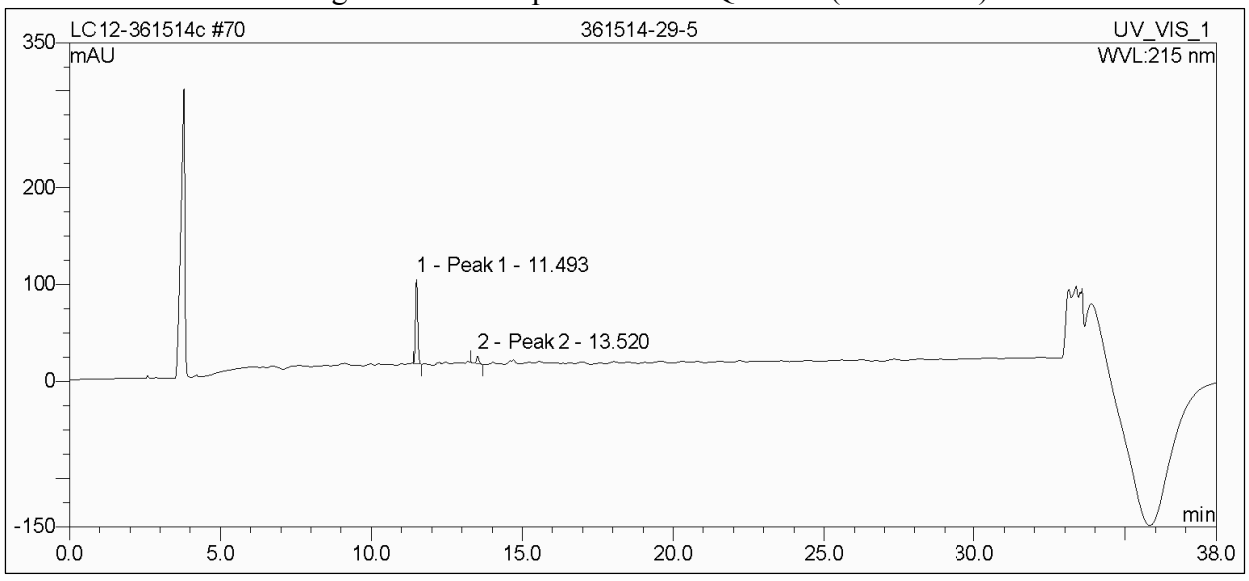
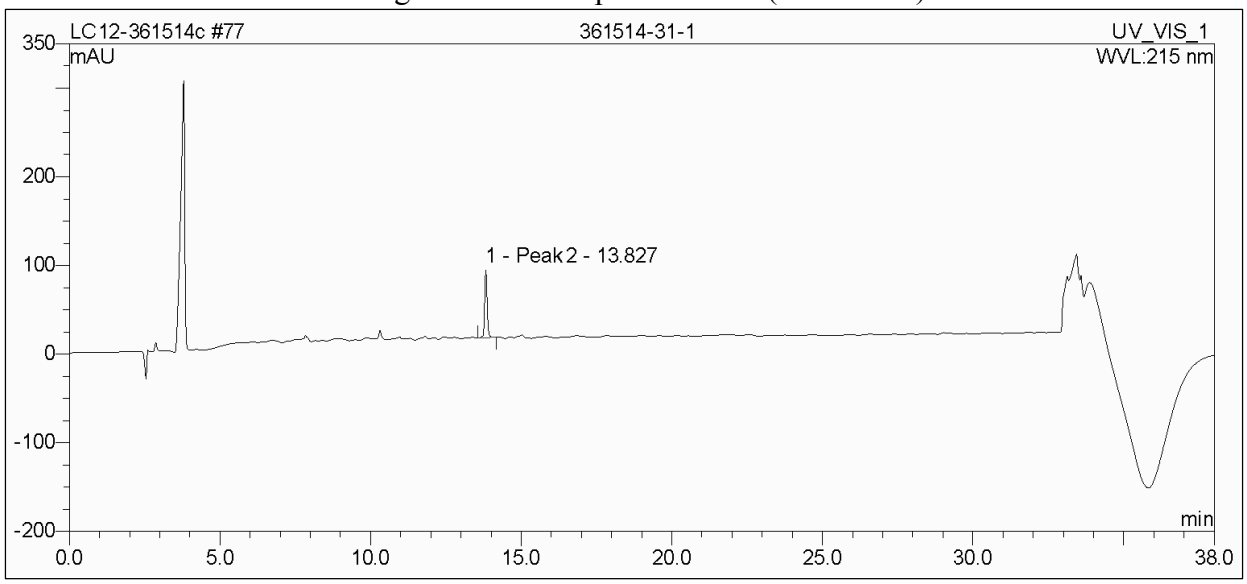


Figure 6. Representative Chromatograms for Linear Peptide Samples Obtained during GI Stability Experiments in SGF

0.5 mg/mL Linear Peptide in SGF (t = 0 hours)



0.5 mg/mL Linear Peptide in SGF (t = 4 hours)

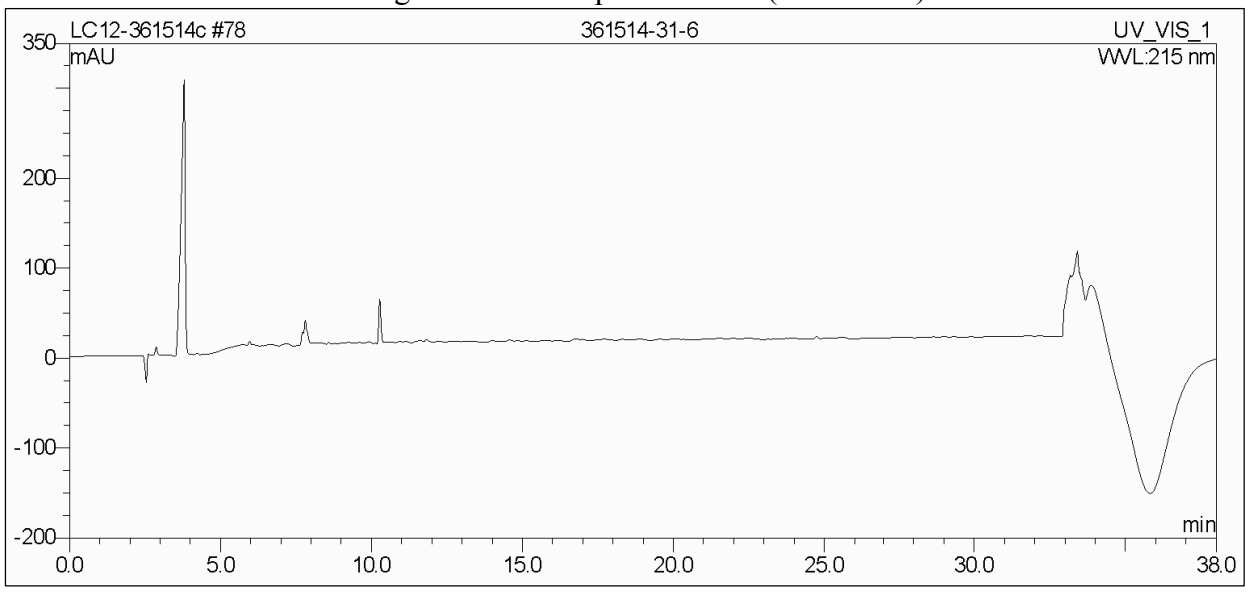
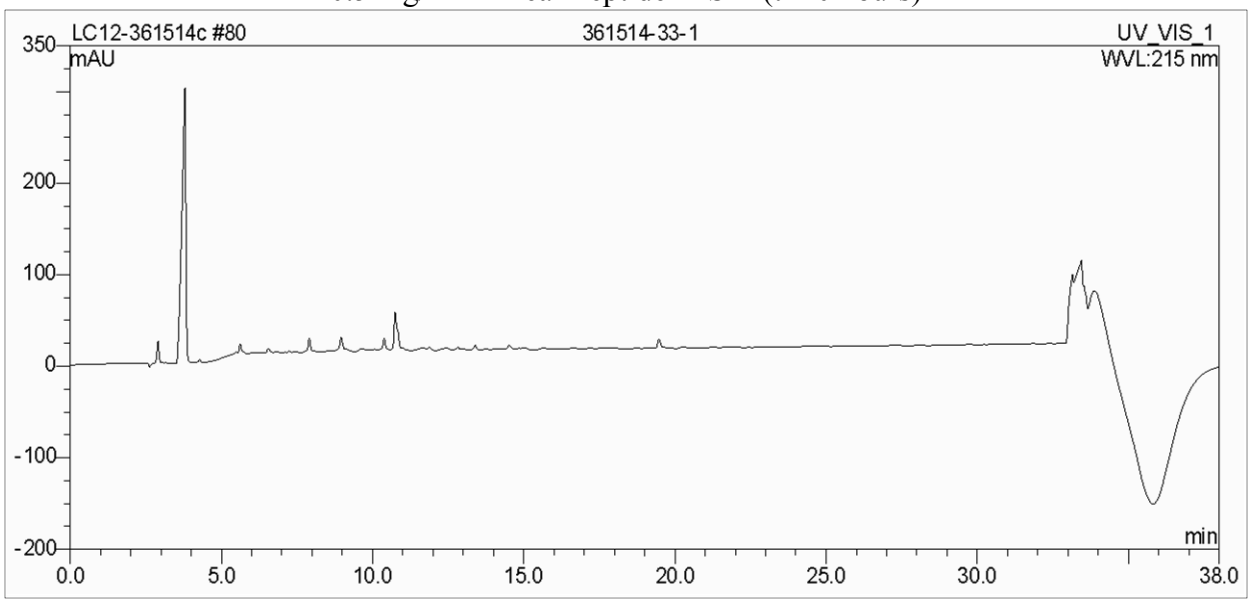
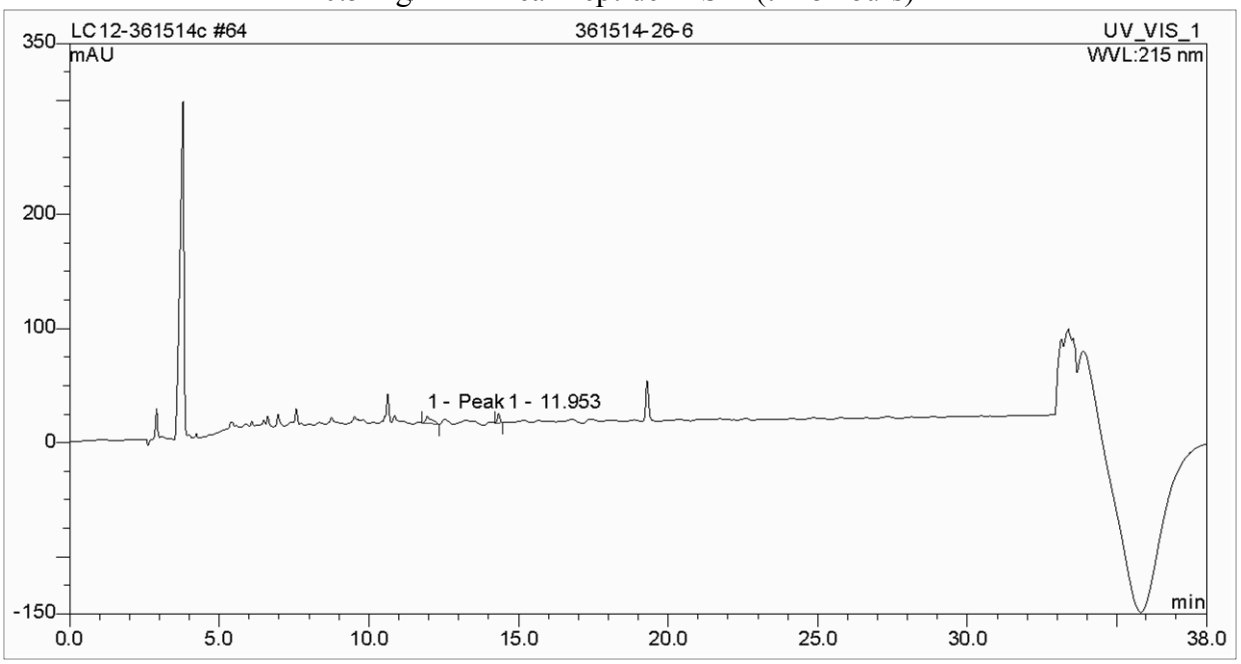


Figure 7. Representative Chromatograms for Linear Peptide Samples Obtained during GI Stability Experiments in SIF

0.5 mg/mL Linear Peptide in SIF (t = 0 hours)



0.5 mg/mL Linear Peptide in SIF (t = 8 hours)



TABLES

Table 1. Back-Calculated Concentrations of the Qualification Calibration Standards (Linear Peptide)

LinearPeptide

Concentration (µg/mL)	**20.0**	**50.0**	**100**
9-Mar-2016 & 10-Mar-2016	17.4	53.2	98.3
	22.7	49.6	102
	18.0	50.3	98.0
Intraset Statistics			
n	3	3	3
Mean	19.4	51.0	99.6
SD	2.9	1.9	2.5
%RSD	15	3.8	2.5
%RE	-3.2	2.1	-0.39

Table 2. Back-Calculated Concentrations of the Qualification Calibration Standards (Cyclic Peptide)

Cyclic Peptide

Concentration (µg/mL)	**20.0**	**50.0**	**100**
9-Mar-2016 & 10-Mar-2016	18.4	51.5	100
	20.2	51.8	98.9
	20.8	47.7	101
Intraset Statistics			
n	3	3	3
Mean	19.8	50.3	99.9
SD	1.2	2.3	0.92
%RSD	6.1	4.6	0.93
%RE	-1.0	0.65	-0.12

Table 3. Calculated Concentrations of the Qualification Quality Control Samples (SGF)

Simulated Gastric Fluid

Concentration (mg/mL)	**0.0**	**0.5**	**0.5**
Peptide	**NA**	**Linear**	**Cyclic**
9-Mar-2016 & 10-Mar-2016	ND	0.406	0.488
	ND	0.429	0.501
Intraset Statistics			
n	2	2	2
Mean	NA	0.417	0.494
SD	NA	0.017	0.0094
%RSD	NA	4.0	1.9
%RE	NA	-17	-1.2

NA=Not Appplicable
ND= Not Detectable

Table 4. Calculated Concentrations of the Qualification Quality Control Samples (SIF)

Simulated Intestinal Fluid

Concentration (mg/mL)	**0.0**	**0.5**	**0.5**
Peptide	**NA**	**Linear**	**Cyclic**
09-Mar-2016 & 10-Mar-2016	ND	0.488	0.499
	ND	0.501	0.491
Intraset Statistics			
n	2	2	2
Mean	NA	0.494	0.495
SD	NA	0.0094	0.0059
%RSD	NA	1.9	1.2
%RE	NA	-1.2	-0.97

NA=Not Appplicable
ND= Not Detectable

Table 5. GI Stability Assessment of Cyclic Peptide in 14-Mar-2016 Formulations (Milli-Q Water)

Group	Time (Hours)	Conc (mg/mL)	Ref # (361514 -)	Run #	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)	Percent of Time Zero (%)
Cyclic (Milli-Q)	0	0.5	24 - 1	113	0.545	109	0.565	0.029	5.2	113	NA
			24 - 2	114	0.586	117					
Cyclic (Milli-Q)	0.5	0.5	24 - 3	115	0.578	116	0.574	0.0050	0.87	115	102
			24 - 4	116	0.571	114					
Cyclic (Milli-Q)	2	0.5	24 - 5	117	0.595	119	0.599	0.0057	0.94	120	106
			24 - 6	118	0.603	121					
Cyclic (Milli-Q)	4	0.5	24 - 7	119	0.608	122	0.560	0.068	12	112	99.0
			24 - 8	120	0.511	102					
Cyclic (Milli-Q)	8	0.5	24 - 9	121	0.551	110	0.537	0.020	3.67	107	95.0
			24 - 10	122	0.523	105					

Analyzed 15-Mar-2016 and 16-Mar-2016

Table 6. GI Stability Assessment of Cyclic Peptide in 14-Mar-2016 Formulations (SGF)

Group	Time	Conc	Ref #	Run #	Analyzed Conc	Percent of Target	Mean Conc	SD	RSD	Mean Conc % of Target	Percent of Time Zero
	(Hours)	(mg/mL)	(361514 -)		(mg/mL)	(%)	(mg/mL)		(%)	(%)	(%)
Cyclic	0	0.5	22 - 1	76	0.561	112	0.568	0.0098	1.7	114	NA
(SGF)			22 - 2	77	0.575	115					
Cyclic	0.5	0.5	22 - 3	78	0.555	111	0.565	0.014	2.5	113	100
(SGF)			22 - 4	79	0.576	115					
Cyclic	1	0.5	22 - 5	80	0.574	115	0.577	0.0038	0.66	115	101
(SGF)			22 - 6	81	0.579	116					
Cyclic	2	0.5	22 - 7	82	0.552	110	0.538	0.020	3.7	108	94.7
(SGF)			22 - 8	83	0.524	105					
Cyclic	4	0.5	21 - 9	92	0.540	108	0.545	0.0082	1.5	109	96.0
(SGF)			21 - 10	93	0.551	110					

Analyzed 15- Mar-2016 and 16-Mar-2016

Table 7. GI Stability Assessment of Cyclic Peptide in 14-Mar-2016 Formulations (SIF)

Group	Time	Conc	Ref #	Run #	Analyzed Conc	Percent of Target	Mean Conc	SD	RSD	Mean Conc % of Target	Percent of Time Zero
	(Hours)	(mg/mL)	(361514 -)		(mg/mL)	(%)	(mg/mL)		(%)	(%)	(%)
Cyclic (SIF)	0	0.5	21 - 11	95	0.581	116	0.548	0.047	8.7	110	NA
			21 - 12	96	0.514	103					
Cyclic (SIF)	2	0.5	21 - 13	97	0.477	95	0.479	0.0028	0.58	95.8	87.5
			21 - 14	105	0.481	96					
Cyclic (SIF)	4	0.5	21 - 15	99	0.429	86	0.409	0.028	6.8	81.9	74.8
			21 - 16	100	0.390	78					
Cyclic (SIF)	6	0.5	21 - 17	101	0.341	68	0.359	0.025	7.0	71.7	65.5
			21 - 18	102	0.376	75					
Cyclic (SIF)	8	0.5	21 - 19	103	0.345	69	0.329	0.023	7.0	65.8	60.1
			21 - 20	104	0.313	63					

Analyzed 15- Mar-2016 and 16-Mar-2016

Table 8. GI Stability Assessment of Linear Peptide in 14-Mar-2016 Formulations (Milli-Q Water)

Group	Time	Conc	Ref #	Run #	Analyzed Conc	Percent of Target	Mean Conc	SD	RSD	Mean Conc % of Target	Percent of Time Zero
	(Hours)	(mg/mL)	(361514 -)		(mg/mL)	(%)	(mg/mL)		(%)	(%)	(%)
Linear (Milli-Q)	0	0.5	29 - 1	131	0.438	88	0.446	0.012	2.7	89.3	NA
			29 - 2	132	0.455	91					
Linear (Milli-Q)	2	0.5	29 - 3	133	0.307	61	0.335	0.040	12	67.0	75.0
			29 - 4	134	0.363	73					
Linear (Milli-Q)	8	0.5	29 - 5	135	0.088	18	0.088	NA	NA	17.6	19.8
			29 - 6	136	ND						

* Linear converted into Cyclic
Analyzed 16- Mar-2016 and 17-Mar-2016
NA= Not Applicable
ND=Not Detectable

Table 9. GI Stability Assessment of Linear Peptide in 14-Mar-2016 Formulations (SGF)

Group	Time	Conc	Ref #	Run #	Analyzed Conc	Percent of Target	Mean Conc	SD	RSD	Mean Conc % of Target	Percent of Time Zero
	(Hours)	(mg/mL)	(361514 -)		(mg/mL)	(%)	(mg/mL)		(%)	(%)	(%)
Linear (SGF)	0	0.5	31 - 1	95	0.418	84	0.413	0.007	1.6	82.6	NA
			31 - 2	96	0.408	82					
Linear (SGF)	2	0.5	31 - 3	97	ND	NA	NA	NA	NA	NA	NA
			31 - 4	105	ND	NA					
Linear (SGF)	4	0.5	31 - 5	99	ND	NA	NA	NA	NA	NA	NA
			31 - 6	100	ND	NA					

Analyzed 16- Mar-2016 and 17-Mar-2016
NA= Not Applicable
ND=Not Detectable

Table 10. GI Stability Assessment of Linear Peptide in 14-Mar-2016 Formulations (SIF)

Group	Time	Conc	Ref #	Run #	Analyzed Conc	Percent of Target	Mean Conc	SD	RSD	Mean Conc % of Target	Percent of Time Zero
	(Hours)	(mg/mL)	(361514 -)		(mg/mL)	(%)	(mg/mL)		(%)	(%)	(%)
Linear	0	0.5	26 - 1	124	ND	NA	NA	NA	NA	NA	NA
(SIF)			26 - 2	125	ND	NA					
			*33 - 1	145	ND	NA					
			*33 - 2	146	ND	NA					
Linear	2	0.5	26 - 3	126	ND	NA	NA	NA	NA	NA	NA
(SIF)			26 - 4	127	ND	NA					
Linear	8	0.5	26 - 5	128	ND	NA	NA	NA	NA	NA	NA
(SIF)			26 - 6	129	ND	NA					

* Back-up samples processed
Analyzed 16- Mar-2016 and 17-Mar-2016
NA= Not Applicable
ND=Not Detectable

APPENDIX A

Study Protocol

PROTOCOL AMENDMENT 1

Study Number: WIL-361514

Sponsor: Promet Therapeutics LLC

Title of Study:

In Vitro Stability of Cyclic (PMT9C151) and Linear (PMT9L152) Peptide in Simulated Gastric and Intestinal Fluids

Protocol Modifications:

Changes are indicated in bold.

1) **3. Study Schedule**

Proposed Draft Letter Report Date: **13 Apr 2016**

Reasons for Protocol Modification:

1) Due to stability concerns, we had to take a stepwise approach while developing analytical method and finalizing experimental design, which also involved significant amount of time trouble shooting. Therefore, there was a delay in experimental completion date, which results in a shift in the draft letter report date. The study update and schedule was promptly communicated with the sponsor.

Approval:

Sponsor approval of the protocol was received by email on 30 Mar2016.

Promet Therapeutics

Sian Bigora
Sponsor Representative

Apr 1, 2016
Date

Jim Noveroske
Sponsor Monitor

Mar. 30, 2016
Date

WIL Research

Prathap N. Shastri, PhD
Study Director

30 Mar 2016
Date

PROTOCOL

In Vitro Stability of Cyclic (PMT9C151) and Linear (PMT9L152) Peptide In Simulated Gastric and Intestinal Fluids

Sponsor:

Promet Therapeutics LLC
7380 Coca Cola Drive Ste 106
Hanover, MD 21076
USA

WIL Study Number: WIL-361514

Sponsor Reference Number: PMT-P-03

Testing Facility:

WIL Research
1407 George Road
Ashland, OH 44805-8946

1 OBJECTIVE:

The objective of this study is to determine the *in vitro* stability of cyclic and linear peptides in simulated gastric and intestinal fluids. This model is used to determine the potential for test article degradation *in vivo* prior to absorption.

This study will be conducted in compliance with the Standard Operating Procedures (SOP) of WIL Research and the protocol as approved by the Sponsor.

2 PERSONNEL INVOLVED IN THE STUDY:

2.1 Sponsor Representative:

Sian Bigora, PharmD
President, Rare Diseases
Promet Therapeutics, LLC
Tel: 4106936844
E-mail: sian.bigora@corlyst-llc.com

2.1 Sponsor Monitor:

Jim Noveroske, PhD
Founder & CSO
Preclindrugdev Consulting LLC
215 Carlene Drive
Sparks, NV 89436
Tel: 775-425-5855
E-mail: jwn@preclindrugdev.com

2.2 WIL Study Director:

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Research Scientist, ADME/DMPK Department
Tel: (419) 289-8700 ext. 3154
Fax: (419) 289-3650
E-mail: prathap.shastri@wilresearch.com

2.3 WIL Departmental Responsibilities:

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E-mail: kerri.hanna@wilresearch.com

Steve Barkyoumb, DVM, PhD, DACVP
Senior Vice-President, U.S. Operations – Nonclinical Safety Assessment

Andy Vick, PhD
Senior Director, Analytical Services

Jennifer Thomas, PhD
Assistant Director, ADME/DMPK

Aimee P. Mahoney, BS
Group Supervisor, Data, Analysis, and Reporting, ADME/DMPK

John S. Moore, BS, LATG
Operations Manager, ADME/DMPK

Robert A. Wally, BS
Operations Manager, Reporting & Technical Support Services

3 STUDY SCHEDULE:

Proposed Experimental Start Date:	25 Feb 2016
Proposed Experimental Termination Date:	25 Feb 2016
Proposed Letter Report Date:	30 Mar 2016

4 TEST ARTICLE:

4.1 Identification:

The test article, cyclic (PMT9C151) and linear (PMT9L152) peptide, will be supplied by or on behalf of the Sponsor. Information concerning the characterization of the test article will be provided by the Supplier and/or Sponsor.

4.2 Physical Description:

To be documented in the study records by WIL Research.

4.3 Spiking Solution Preparation:

The spiking solutions will be prepared by solubilizing test article in water and diluting as required. Details of the preparation of the spiking solutions will be included in the study records and described in the final report. The final vehicle concentration will be approximately ≤10% v/v in Simulated Gastric Fluid (SGF)

or Simulated Intestinal Fluid (SIF). The spiking solution will be prepared immediately prior to addition to the SGF and SIF.

4.4 Dose Formulation Analysis:

Spiking Solution analysis will not be performed.

4.5 Storage Conditions:

The test article will be stored at approximately -20°C when not in use.

4.6 Reserve Samples:

As this is a non-regulated study, no reserve samples will be collected.

4.7 Personnel Safety Data:

Appropriate safe handling procedures will be used based on available information for the test articles.

5 METABOLISM IN WATER AND SIMULATED DIGESTIVE FLUIDS:

5.1 Overview:

Simulated gastric and intestinal fluids (SGF and SIF, respectively) will be prepared according to the instructions in the United States Pharmacopeia (USP 36). Cyclic (PMT9C151) and linear (PMT9L152) peptides (500 µM) will be incubated independently in aliquots of SGF and SIF with digestive enzymes. Control incubations in Milli-Q water will be run concurrently. The aliquots will be flash frozen at selected time points for analysis by HPLC-UV.

5.2 In Vitro Phase:

5.2.1 Simulated Gastric Fluid:

Simulated gastric fluid will be prepared according to the directions in the USP 36:

"Dissolve 2.0 g of sodium chloride and 3.2 g of purified pepsin that is derived from porcine stomach mucosa, with an activity of 800 to 2500 nits per mg of protein, in 7.0 mL of hydrochloric acid and sufficient water to make 1000 mL. This test solution has a pH of about 1.2"

The preparation of SGF may be scaled as necessary. Certificate of Analysis will be obtained for the pepsin, and the amount of pepsin used for SGF preparation will be calculated to meet the USP units requirement. In addition, the amount of salt and enzyme used may be adjusted during

the SGF preparation to ensure that the final vehicle concentration is maintained at ≤10% v/v in SGF as described in section 4.3.

5.2.2 Simulated Intestinal Fluid:

Simulated intestinal fluid will be prepared according to the directions in the USP 36:

"Dissolve 6.8 g of monobasic potassium phosphate in 250 mL of water, mix, and add 77 mL of 0.2 N sodium hydroxide and 500 mL of water. Add 10.0 g of pancreatin, mix, and adjust the resulting solution with either 0.2 N sodium hydroxide or 0.2 N hydrochloric acid to a pH of 6.8 ± 0.1. Dilute with water to 1000 mL.

The preparation of SIF may be scaled as necessary. Certificate of Analysis will be obtained for the pancreatin, and the amount of pancreatin used for SIF preparation will be calculated to meet the USP units requirement. In addition, the amount of salt and enzyme used may be adjusted during the SIF preparation to ensure that the final vehicle concentration is maintained at ≤10% v/v in SIF as described in section 4.3.

5.2.3 Test Article Incubations:

The stability of cyclic (PMT9C151) and linear (PMT9L152) peptides will each be evaluated at a concentration of 500 µg/mL in Millli-Q water, SGF (with enzymes), and SIF (with enzymes). Incubations will be performed in capped glass vials in a shaking water bath at 37ºC. Duplicate vials (0.5 mL each) of test article in water or simulated fluids will be prepared for each time point. At the appropriate time points (0, 0.5, 2, 4, and 8 for cyclic and 0, 2, and 8 for linear peptide), water reactions will be divided into approximately equal aliquots and the samples will be flash frozen and stored at -70ºC until analysis. At the appropriate time points (0, 0.5, 1, 2, and 4 hours for cyclic peptide, and 0, 2, and 4 hours for linear peptide), SGF reactions (with enzymes) will be divided into approximately equal aliquots and the samples will be flash frozen and stored at -70ºC until analysis. At the appropriate time points (0, 2, 4, 6, and 8 for cyclic peptide and 0, 2, 8 for linear peptide) SIF reactions (with enzymes) will be divided into approximately equal aliquots and the samples will be flash frozen and stored at -70ºC until analysis. The final methods will be documented in the study records.

5.3 Sample Analysis:

An HPLC-UV method adapted from the method provided by the Sponsor will be used to quantify the degree of degradation of cyclic (PMT9C151) and linear (PMT9L152) peptides in the samples. Cyclic peptide incubations will be analyzed for the concentrations of cyclic peptide, and linear peptide incubations will be analyzed for the concentrations of linear peptide. In addition, cyclic peptide incubations may be analyzed for the presence of linear peptide, and linear peptide incubations may be analyzed for the presence of cyclic peptide. The final methods will be documented in the study records.

6 STATISTICAL METHODS:

Descriptive statistics (e.g., totals, arithmetic means, standard deviations, standard errors, coefficients of variation, percentages) generated in the study will be performed using programs of the WIL Toxicology Data Management System or spreadsheets according to WIL SOPs. Where appropriate, figures will be generated using Prism software (GraphPad Software, Inc., San Diego, CA).

7 QUALITY ASSURANCE:

The raw data generated by WIL and draft letter report will be reviewed by WIL Quality Control personnel to assure that the final summary report accurately describes the conduct and the findings of the study.

This is a non-regulated study and will not 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, will be stored as described in Work Product protocol section.

9 WORK PRODUCT:

Sponsor will have title to all documentation records, raw data, specimens, or other work product generated during the performance of the study. All work product including paper raw data and pertinent electronic storage media, but excluding specimens (e.g. samples collected during the experimental design phase of the study), will be retained at no charge for a period of six months following issuance of the final report in the Archives at WIL Research. Thereafter, WIL Research will charge a monthly archiving fee for retention of all work product. All work product will be stored in compliance with regulatory requirements. Specimens will be discarded after the issuance of the final report. WIL Research will retain any remaining unused test article(s) until their stated expiry date, but no longer than 3 years after the issuance of the final report. After this period, the test article(s) will be destroyed.

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

10 REPORTS:

Following conclusion of experimental activities, WIL Research will prepare a letter report that will contain the information generated during the conduct of the study.

WIL Research will submit 1 electronic copy (*e.g.,* PDF) and a MS Word version of an unaudited draft letter report in a timely manner upon completion of data collection prior to issuance of the final report. One (1) revision will be permitted as part of the cost of the study, from which Sponsor's Representative's reasonable revisions and suggestions will be incorporated into the Final Letter Report as appropriate. Additional changes or revisions may be made, at extra cost. It is expected that the Sponsor will review the draft letter report and provide comments to WIL within two months. WIL will submit the Final Letter Report within one (1) month following receipt of comments. Two (2) electronic copies of the Final Letter Report will be provided: requests for additional copies of the Final Letter Report may result in additional charges.

11 PROTOCOL MODIFICATION:

Modification of the Protocol may be accomplished during the course of this investigation. 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. The Sponsor will be informed of all deviations from the Protocol. Protocol deviations will be documented in the final report.

12 REFERENCES:

The United States Pharmacopeia (36), The National Formulary (31). United States Pharmacopeial Convention, Inc., Rockville, MD 2013.

13 PROTOCOL APPROVAL:

Sponsor approval of the protocol was received by email on 24 February 2016.

Promet Therapeutics

Sian Bigora, PharmD
Sponsor Representative

Mar 14, 2016
Date

Jim Noveroske, PhD
Sponsor Monitor

Mar. 11, 2016
Date

WIL Research

Prathap N. Shastri, PhD
Study Director

24 Feb 2016
Date

APPENDIX B

Test Material Information

SIGMA-ALDRICH® sigma-aldrich.com

3050 Spruce Street, Saint Louis, MO 63103, USA
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:
Pepsin from porcine gastric mucosa – lyophilized powder, 3,200–4,500 units/mg protein

Product Number:	**P6887**
Batch Number:	**SLBM3033V**
Brand:	SIGMA
CAS Number:	9001-75-6
MDL Number:	MFCD00081840
Storage Temperature:	Store at -20 ℃
Quality Release Date:	19 DEC 2014
Date Retested:	28 JUL 2015
Recommended Retest Date:	JUL 2016

Test	Specification	Result
Appearance (Color)	White to Off White	Off-White
Appearance (Form)	Powder	Powder
% Protein (UV)	70 - 100	96
units/mg Protein One unit will produce a change in A280 of 0.001 per min at pH 2.0 at 37 deg C, measured as TCA-soluble substrate using hemoglobin as substrate. (Final volume = 16 mL, Light Path = 1 cm)	3200 - 4500	3913

Rodney Burbach, Manager
Analytical Services
St. Louis, Missouri US

SIGMA-ALDRICH® sigma-aldrich.com

3050 Spruce Street, Saint Louis, MO 63103, USA
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:
Pancreatin from porcine pancreas - powder, suitable for cell culture, 4 × USP specifications

Product Number:	**P3292**
Batch Number:	**SLBN4829V**
Brand:	SIGMA
CAS Number:	8049-47-6
MDL Number:	MFCD00131789
Storage Temperature:	Store at -20 ℃
Quality Release Date:	12 JUN 2015

Test	Specification	Result
Appearance (Color)	Light Yellow to Tan	Light Tan
Appearance (Form)	Powder	Powder
Cell Dissociation Assay The cell count should not be less than a two-fold increase when compared to the 24 hour count.	Pass	Pass
Digestion Power 4X USP specifications	Conforms	Conforms

Rodney Burbach, Manager
Analytical Services
St. Louis, Missouri US

APPENDIX C

Laboratory Method

Analytical Method for the Analysis of PMT9C151 (cyclic) and PMT9L152 (linear) in Aqueous Formulations by HPLC/UV

1. PURPOSE

The purpose of this method is to describe procedures to be employed for the analysis of PMT9C151 and PMT9L152 in aqueous formulations by HPLC/UV.

2. SCOPE

The procedures provided in this method are applicable for the quantitation of PMT9C151 and PMT9L152 in aqueous formulations at a concentration of 500 µg PMT9C151/mL and 500 µg PMT9L152/mL.

3. DEFINITIONS/ABBREVIATIONS

The following abbreviations may appear in this method:

µL	-	microliter
µg	-	microgram
ACN	-	acetonitrile
cm	-	centimeter
CAD	-	charged aerosol detector
CMC	-	carboxymethylcellulose
DAD	-	diode array detector
DI	-	deionized
DMSO	-	dimethylsulfoxide
ECD	-	electron capture detector
EtOH	-	ethanol
FA	-	formic acid
FID	-	flame ionization detector
GAA	-	glacial acetic acid
GC	-	gas chromatography
HPLC	-	high performance liquid chromatography
HPMC	-	hydroxypropyl methylcellulose
IC	-	ion chromatography
IS	-	internal standard
kg	-	kilogram
L	-	liter
M	-	molar
MC	-	methylcellulose

MeOH	-	methanol
mg	-	milligram
mL	-	milliliter
mm	-	millimeter
mM	-	millimolar
MS	-	mass spectrometry
NA	-	not applicable
ng	-	nanogram
nm	-	nanometer
ppm	-	parts per million
pg	-	picogram
QC	-	quality control
%RE	-	percent relative error
RI	-	refractive index detector
RSD	-	relative standard deviation
SD	-	standard deviation
TFA	-	trifluoroacetic acid
UHPLC	-	ultra-high performance liquid chromatography
UV	-	ultraviolet
v	-	volume
VIS	-	visible
VWD	-	variable wavelength detector
w	-	weight

4. EQUIPMENT AND SUPPLIES

The following equipment and/or supplies may be used while performing this method:

96-well analytical plates
Analytical balances and weighing vessels
Autosampler vials and caps with appropriate liners
Class A glass pipettes
Corning® Costar® 3635, acrylic 96-well UV plates
Disposable pipettes
Laboratory glassware (*e.g.*, volumetric flasks, graduated cylinders, beakers, *etc.*)
Laboratory refrigerators, freezers, incubators, *etc.*
Laboratory sample mixing equipment (shakers, vortexers, *etc.*)
Membrane filters of 0.45-µm (or finer) porosity
pH meters
Polypropylene labware (*e.g.*, volumetric flasks, graduated cylinders, beakers, *etc.*)
Polypropylene tubes and caps with appropriate liners
Repeater pipettes with appropriately sized tips
Sonicators
Syringe-end filters of 0.45-µm (or finer) porosity
Syringes with dosing cannula or needles

5. PROCEDURE

5.1. PREPARATION OF REAGENTS

Volumes of these reagents can be adjusted as long as proportionality is maintained and their preparation is documented in the study records. Expiration dates and storage conditions of prepared reagents will be assigned according to WIL Research SOPs.

5.1.1. 1:100 (V/V) TFA:MILLI-Q WATER (DILUENT)

Combine TFA and Milli-Q water in a 1:100 (v/v) ratio and stir to mix.

5.2. PREPARATION OF STOCK SOLUTIONS

The following preparation schemes are suggested approaches. Appropriate modifications to reach the targeted nominal calibration and QC concentrations are acceptable. For example, if the concentration of a primary stock solution is not practical for use in the preparation of calibration

standards or QC samples, a secondary stock solution may be prepared. The preparation of any secondary or working stock solutions will be documented in the study records. Volumes of these stock solutions can be adjusted as long as proportionality is maintained and their preparation is documented in the study records. Expiration dates and storage conditions of stock solutions are assigned based on available stability data.

Stock solutions are corrected for purity, water content, and salt content, if applicable.

5.2.1. PREPARATION OF STANDARD A AND B SAMPLES

The PMT9C151 and PMT9L152 stock solutions A and B are prepared at a concentration of 0.100 mg PMT9C151/mL and 0.100 mg PMT9C151/mL as follows. Weigh approximately 1.0 mg of PMT9C151 and PMT9L152 in a tared glass weigh funnel and transfer to a 10-mL volumetric flask with rinses of diluent. Add approximately 75% of the final volume of diluent to the preparation and mix the preparation as necessary to achieve complete dissolution. Add additional diluent to yield the desired concentration, and thoroughly mix the solution.

Standard A and B samples are prepared at a concentration of 50.0 µg PMT9C151/mL and 50.0 µg PMT9C151/mL by combining aliquots of the appropriate stock solution and diluent in amber autosampler vials. The samples are mixed with vortex action.

5.2.2. PREPARATION OF CALIBRATION STOCK SOLUTION

For any qualification sessions, a PMT9C151 and PMT9L152 calibration stock solution is prepared at a concentration of 0.100 mg PMT9C151/mL and 0.100 mg PMT9L152/mL as follows. Weigh approximately 1.0 mg of PMT9C151 and PMT9L152 in a tared glass weigh funnel and transfer to a 10-mL volumetric flask with rinses of diluent. Add approximately 75% of the final volume of diluent to the preparation and mix the preparation as necessary to achieve complete dissolution. Add additional diluent to yield the desired concentration, and thoroughly mix the solution.

5.2.3. PREPARATION OF QUALITY CONTROL STOCK SOLUTION

For any qualification sessions, a PMT9C151 and PMT9L152 calibration stock solution is prepared at a concentration of 0.100 mg PMT9C151/mL and 0.100 mg PMT9L152/mL as follows. Weigh approximately 1.0 mg of PMT9C151 and PMT9L152 in a tared glass weigh funnel and transfer to a 10-mL volumetric flask with rinses of diluent. Add approximately 75% of the final volume of diluent to the preparation and mix the preparation as necessary to achieve complete dissolution. Add additional diluent to yield the desired concentration, and thoroughly mix the solution.

5.3. PREPARATION OF CALIBRATION SAMPLES

Prepare calibration samples at concentrations ranging from 20.0 and 100 µg PMT9C151/mL and 20.0 and 100 µg PMT9L152/mL by combining aliquots of the calibration stock solution, and diluent. Prepare at least triplicate calibration samples at each concentration for any qualification sessions. The samples are mixed with vortex action.

5.4. PREPARATION AND PROCESSING OF QC SAMPLES

For any qualification sessions, prepare QC samples to simulate the processing of formulations by combining aliquots of the appropriate QC stock solution, vehicle, and diluent in polypropylene tubes. Mix the samples with vortex action, inversion, shaking, and/or sonication. Further dilute the QC samples as needed to achieve a final diluted concentration within the calibration range, preferably at the same concentration as the standard samples by combining aliquots of the appropriate samples, vehicle, and diluent in polypropylene tubes. The samples are mixed with vortex action. Prepare the QC samples in triplicate at each concentration; prepare a single vehicle blank sample. Alternate QC concentrations may be prepared within the validated range and using the same general dilution schemes.

5.5. FORMULATION SAMPLE COLLECTION AND PROCESSING

Collect samples from each formulation using a syringe and dosing cannula and place in polypropylene tubes. Process at least 2 samples from each set for analysis; the remaining

samples (back-up samples) are stored as indicated in the protocol or according to WIL Research SOP if not designated in the protocol and, if not needed for analysis, will be discarded as indicated in the protocol or according to WIL Research SOP if not designated in the protocol. Process the formulation samples by adding diluent and mix with vortex action. Further dilute portions of the processed samples as needed to achieve a final diluted concentration within the calibration range, preferably at the same concentration as the standard samples, by combining aliquots of the appropriate samples, and diluent in polypropylene tubes. The samples are mixed with vortex action.

5.6. INSTRUMENTATION

Instrumentation can be substituted provided that the design parameters of the substituted instrumentation are at least comparable to those of the unit initially used.

5.6.1. HPLC SET-UP PARAMETERS (GRADIENT)

Instrument:	Agilent 1100 liquid chromatograph equipped with a variable wavelength detector, autosampler, and Dionex Chromeleon® software version 6.8, or equivalent system
Column:	YMC-Pack ODS-AM, 250 mm × 4.6 mm, 5-µm particle-size
Mobile Phase:	A: 1000:1 (v/v) DI water:TFA B: 1000:1 (v/v) ACN:TFA
Flow Rate:	1.00 mL/minute

Gradient:

Time (minutes)	A (%)	B (%)
0	98	2
30.0	68	32
30.1	1	99
32.0	98	2
38.0	98	2

Column Temperature:	Ambient
Autosampler Temperature:	Ambient
Detector:	UV at 215 nm
Injection Volume:	20 µL
Retention Time:	Approximately 11.6 minutes for PMT9C151 (cyclic), and 13.7 minutes for PMT9L152 (linear)
Run Time:	38.0 minutes

5.7. LINEARITY ASSESSMENT QUANTITATION

Single injections are made of each processed calibration, QC, standard, and formulation sample. A calibration curve is constructed for each set of analyses. The PMT9C151 and PMT9L152 peak areas (y) and the theoretical concentrations (x) of the calibration samples are fit with least-squares regression analysis to the linear function forced through the origin, without weighting:

$$y = ax + b$$

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

The analytical results are evaluated against acceptance criteria detailed in the protocol and/or WIL Research SOPs AC-047 or AC-048. Deviations from expected results are investigated according to the appropriate departmental SOP.

Data is reported to the Principal Chemist, the Study Director, and other appropriate individuals.

5.8. QUANTITATION, ACCEPTANCE CRITERIA, AND DATA REPORTING

Quantitation is performed using the response factor technique, *i.e.*, calculating the response factor from the peak areas of injections of samples with known PMT9C151 and PMT9L152 concentrations. The PMT9C151 and PMT9L152 response factor of the standard sample (in this case standard A sample) is calculated as follows:

Response Factor = PMT9C151 and/or PMT9L152 Peak Area / PMT9C151 and/or PMT9L152 Concentration

The concentration of PMT9C151 and/or PMT9L152 in the samples is calculated by dividing the sample peak area by the average standard response factor and multiplying by the multiplication factor. The multiplication factor corrected for sample dilution.

Concentration = (Peak Area Mean/Standard Response Factor) × Multiplication Factor

Concentrations are calculated from the results of the regression analysis using Microsoft Excel®. The concentration data are transferred to a Microsoft Excel® spreadsheet where appropriate summary statistics, *i.e.*, mean, SD, RSD, and concentration as a percent of target concentration, were calculated and presented in tabular form. The concentrations of the formulations samples were calculated by applying any necessary factors to correct for dilution and/or unit conversion.

The analytical results are evaluated against acceptance criteria detailed in the protocol and/or WIL Research SOP-AC-047 or SOP-AC-048. Deviations from expected results are investigated according to the appropriate WIL Research departmental SOP.

Data is reported to the Principal Chemist, the Study Director, and other appropriate individuals.

5.9. ASSAY ACCEPTABILITY FOR ROUTINE ANALYSES

In addition to the experimental samples, each analytical session consists of (but is not limited to) duplicate standard samples at a single concentration. For an analytical session to be considered valid, the test article peak areas from 6 replicate injections of standard A sample must have an RSD ≤2.0%. Also, the difference in concentration in standard A and B samples is within

98% and 102% of the target concentration. In addition, the test article peak area response from the initial standard A sample injections and the periodic injections of standard A sample (injection performed after every 6 sample injections as well as at end of sequence) must have an RSD ≤2.0%. The peak asymmetry must be ≤2.0. Any analytical session that does not meet these criteria will be investigated to determine the impact, if any, on the results.

5.10. QUALIFICATION

The PMT9C151 and PMT9L152 assay procedure was qualified in this study with a single qualification session. Quantitation was performed using calibration standards ranging from 20.0 to 100 µg/mL. The intra-session variability (RSD) and %RE of the mean back-calculated standard concentrations of the calibration standards prepared for the qualification are summarized in the following table.

Qualification	RSD Range of Values (%)	%RE Range of Values (%)
Cyclic Peptide	0.93 to 6.1	-1.0 to 0.65
Linear Peptide	2.5 to 15	-3.2 to 2.1

The results met the WIL Research SOP acceptance criteria for calibration standards, *i.e.*, RSD ≤10% and %RE within ± 10% (except at the lowest calibration level where RSD ≤15% and %RE within ± 15% were acceptable).

Assay precision and accuracy were verified by the analysis of QC samples. The intra-session variability (precision) and %RE (accuracy) of the mean calculated QC concentrations of the samples prepared for the qualification, are summarized in the following table.

Qualification	Matrix	Target Concentration (mg/mL)	RSD Values (%)	%RE Values (%)
Linear	SGF	0.500	4.0	-17
Cyclic	SGF	0.500	1.9	-1.2
Blank	SGF	0.00	NA	NA

Linear	SIF	0.500	1.9	-1.2
Cyclic	SIF	0.500	1.2	-0.97
Blank	SIF	0.00	NA	NA

The results met the WIL Research SOP acceptance criteria for precision and accuracy, *i.e.*, RSD ≤10% and %RE within ± 10% with an exception of the linear peptide in SGF matrix had a %RE value of -17.

6. VALIDATION HISTORY/STABILITY PARAMETERS/REFERENCES/SYNONYMS

Non-GLP Method Qualification in WIL-361514

7. REVISION HISTORY

NA