A Sensitive LC-MS/MS Method for Determination of Ipamorelin in Human Plasma

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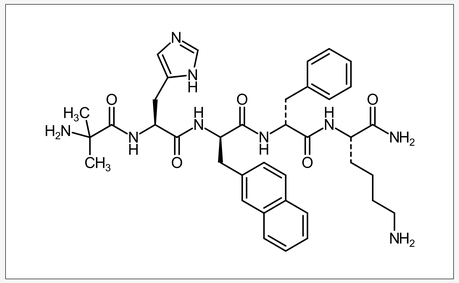
INTRODUCTION

Post-operative gastrointestinal (GI) dysmotility, including ileus, remains a clinical problem. Ghrelin, a 28-amino acid peptide hormone, is primarily synthesized in the oxyntic gland in the stomach. It can modulate gastric motility and gastric acid secretion and in a rat model ghrelin has been shown to resolve gastric post-operative ileus. Ipamorelin is a ghrelin receptor agonist and is available as an intravenous treatment, the preferred route of administration for patients unable to tolerate oral administration. The purpose of this work was to develop and validate a sensitive and reliable method with sufficient matrix stability data for determination of ipamorelin in human plasma to support the Phase II clinical study HT-IPAM-202.



Ipamorelin (MW 711.86)

METHOD

A protein precipitation procedure was used for sample cleanup. Ipamorelin and a stable isotope labeled internal standard (ipamorelin-$^{13}C_6$, $^{15}N_2$; MW=719.81) in the resulting supernatant were chromatographed using a valve switching system consisting of a C18 column (Figure 1) for reducing the carryover. Ipamorelin and its internal standard were detected by API 4000 LC/MS/MS system under positive MRM mode.

Liquid Chromatography

HPLC : Shimadzu 10ADvp
Autosampler : Perkin Elmer 200
Column: Pursuit C18 (50x2 mm) by Varian
MP A : 0.1% Formic acid in DI H_2O
MP B : MeOH/ACN/Formic acid (60/40/0.1)
MP C : ACN/DI H_2O (90/10)

Mass Spectrometry

AB Sciex API 4000
Polarity : Positive
Scan type : Multiple Reaction Monitoring
Ipamorelin: m/z 357.0 → 129.0
Ipamorelin-$^{13}C_6$, $^{15}N_2$: m/z 361.0 → 137.0 (I.S.)
(Parent ions are 2+ charged.)

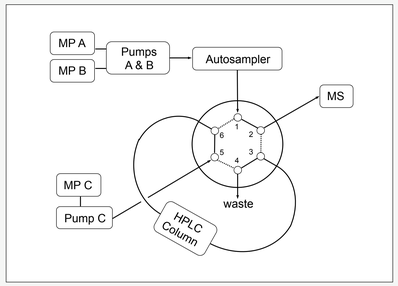


Figure 1. Diagram for LC/MS Instruments

RESULTS

The method showed a linear range of 5 to 960 ng/mL with weighted linear regression (1/x). The correlation coefficient was 0.995 or better (Figure 2).

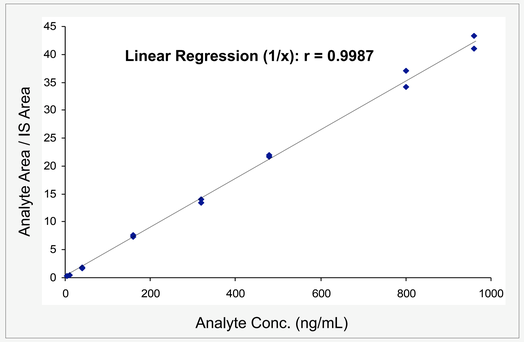


Figure 2. A Typical Calibration Curve for Ipamorelin

Figures 2 through 4 show typical chromatograms for selected samples. The S/N ratio for ipamorelin peak at LLOQ level was greater than 30 .

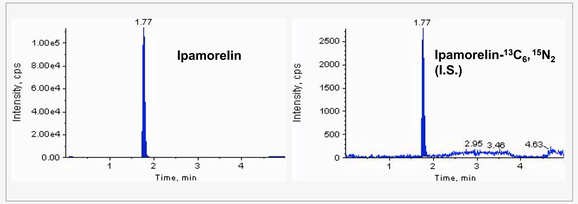


Figure 2. Chromatogram of an Extracted ULOQ Sample (960 ng/mL).

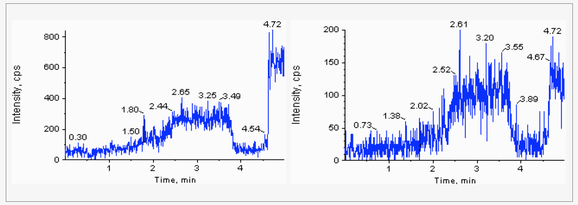


Figure 3. Chromatogram of a Reconstitution Solvent Blank Injected after ULOQ

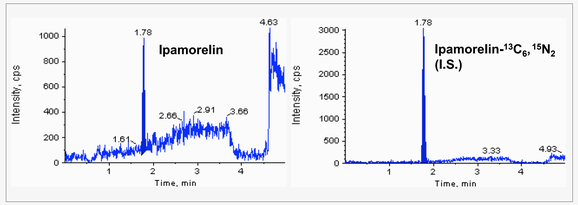


Figure 4. Chromatogram of an Extracted LLOQ sample (5 ng/mL).

The method is robust for the analysis of ipamorelin in human plasma including lipemic and hemolytic samples (Table 1). Ipamorelin is stable in human blood stored in ice-water bath for up to 1 hour. The matrix stability of ipamorelin in human K_2EDTA plasma was also established for four freeze/thaw cycles when frozen at either -20ºC or -70ºC and thawing at either room temperature or in the 37ºC water bath. Iparmorelin in human plasma was stable at room temperature for at least 6 hours and at -70 ºC for at least 216 days.

	Hemolytic	Lipemic
mean	**14.2**	**15.3**
n	**6**	**6**
SD	**0.492**	**1.02**
% CV	**3.5**	**6.7**
% Bias	**-5.6**	**2.1**

Table 1. Hemolytic and Lipemic Matrix Evaluation Using Low QC samples (15 ng/mL).*

* These data were obtained using D-Ala-β-(2-naphthyl)-D-Ala-Trp-D-Phe-Lys amide (MW 746.90) as internal standard (Sigma# A2960).

The intra-batch accuracy (%Bias) for above LLOQ QCs ranged from –3.7 to 5.3% with a precision (CV%) range of 3.1 to 7.0% (Table 2). The inter-batch accuracy ranged from –0.7 to 3.6% with a precision range of 5.5 to 6.0%. Matrix effect was evaluated for ipamorelin in six different lots of blank human plasma at concentrations of 15.0 ng/mL with a variation of response (CV%) at 3.6% (Table 3).

Batch		**5.00** ng/mL (LLOQ)	**15.0** ng/mL (Low)	**240** ng/mL (Mid)	**640** ng/mL (High)
1	**Mean**	5.15	15.5	236	672
	% CV	13.1	6.9	3.1	4.0
	% Bias	2.9	3.2	-1.5	5.0
2	**Mean**	4.57	15.6	247	674
	% CV	14.5	5.5	7.0	6.2
	% Bias	-8.7	3.9	2.9	5.3
3	**Mean**	4.65	15.4	231	643
	% CV	11.7	4.9	6.0	5.9
	% Bias	-6.9	2.5	-3.7	0.5
Overall	**Mean**	4.79	15.5	238	663
	n	18	18	18	18
	% CV	13.5	5.5	6.0	5.6
	% Bias	-4.2	3.2	-0.7	3.6

Table 2. Precision and Accuracy Results for Ipamorelin Quality Control Samples (n = 6 replicates per batch).*

	Avg drug area	Avg I.S. area	Avg area ratio
Lot 1	4617	67032	0.06887
Lot 2	4775	70321	0.06790
Lot 3	4493	67733	0.06634
Lot 4	4774	64928	0.07353
Lot 5	4642	66377	0.06994
Lot 6	4519	66288	0.06818
mean	**4637**	**67113**	**0.06913**
n	**6**	**6**	**6**
SD	**121**	**1827**	**0.00246**
%CV	**2.6**	**2.7**	**3.6**

Table 3. Matrix Effect for Ipamorelin in Human Plasma.*

CONCLUSION

This method showed acceptable accuracy, precision, selectivity, stability, and reproducibility, and was validated for the determination of ipamorelin in human plasma. The method is simple and reliable for the quantitation of ipamorelin and adequate to support further drug development and clinical studies.

ACKNOWLEDGEMENTS

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