

## INTRODUCTION

Collection of samples for clinical bioanalysis is typically conducted by a trained phlebotomist at a clinic or hospital and requires a painful blood draw and large volumes of blood. This makes pediatric blood sampling difficult and can be burdensome for the elderly or critically ill. The Tasso-M20 device enables at-home, automated self-collection of blood samples. The device automatically collects 17.5 microliter samples in minutes with very little pain or blood loss. BPC-157 is a pentadecapeptide gastric peptide that possesses free radical scavenging activity and has been shown to reduce inflammation by blocking the production of pro-inflammatory mediators (nitric oxide, prostaglandins, and leukotrienes). BPC-157 has been shown to have healing properties for gastrointestinal fistulas, intestinal lesions, and liver lesions and can be used for the treatment of inflammatory bowel disease and congestive heart failure. BPC-157 has also been shown to accelerate the healing of damaged tendons and ligaments. In order to accurately quantify the peptide in blood collected on the Tasso device an extraction and HPLC-MS/MS method was developed with a dynamic range of 1.00-2000 ng/mL.

## OVERVIEW

### Purpose

- Develop an Accurate/Precise HPLC-MS/MS method for the Quantitation the Pentadecapeptide BPC-157 from Human Blood collected with a Tasso-M20 Device by HPLC-MS/MS.

### Methods

- BPC-157 was extracted from dried human blood collected on a Tasso-M20 device and analyzed using HPLC-MS/MS.

### Results

- The developed method resulted in a sensitive, selective assay that can be utilized to analyze BPC-157 from dried human blood samples collected with a Tasso-M20 device with an LLOQ of 1.00 ng/mL.

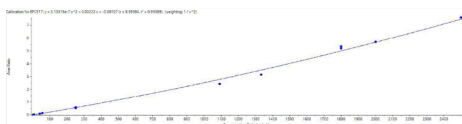


Figure 1: Regression Line of BPC-157 Standard Curve Analyzed from Extracted Dried Blood Samples

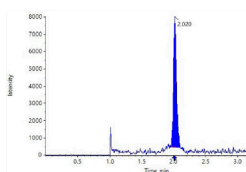


Figure 2: Extracted Dried Human Blood Chromatogram 1.00 ng/mL

## METHODS

### Extraction

- Add 17.5  $\mu$ L of blood to each absorbent tip and allow to dry for at least 4 hours
- Add dried absorbent tip + 25  $\mu$ L Internal Standard into a 96 DWP
- Add 0.100 mL water to each well and allow to soak for 30 minutes
- Add 500  $\mu$ L ACN to each well, vortex, centrifuge
- Remove 400  $\mu$ L supernatant
- Dry wells using nitrogen
- Add 0.100 mL water with 0.1% formic acid, vortex 5 min, centrifuge briefly
- Inject onto the HPLC-MS/MS

### HPLC Parameters

- Column: Agilent Pursuit 5 Diphenyl, 100X2.1 mm
- LC Pumps: Shimadzu LC-30AD
- MP A: Water 0.1% Formic Acid
- MP B: ACN 0.1% Formic Acid
- Flowrate: 0.700 mL/min
- LC Gradient: 95% A to 70% A at 3 minutes

### Mass Spectrometry

- SCIEX 7500
- Positive ion mode
- MRM

Standard Concentration	Sample Type	Calc. Conc. (ng/mL)	Accuracy (%)
1.00	Standard	1.07	107
1.00	Standard	0.944	94.4
2.00	Standard	1.85	92.5
2.00	Standard	2.10	105
8.00	Standard	8.17	102
8.00	Standard	7.52	94.0
50.0	Standard	54.4	109
50.0	Standard	54.4	109
250	Standard	263	105
250	Standard	232	92.8
1000	Standard	1060	106
1000	Standard	1090	109
1800	Standard	1900	106
1800	Standard	1870	104
2000	Standard	2030	102
2000	Standard	2010	101

Table 1: Accuracy of BPC-157 Extracted from Human Dried Blood



Image 1: Tasso-M20 in use, [tassoinc.com](http://tassoinc.com)

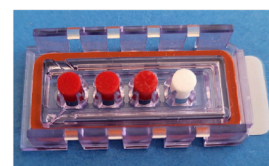


Image 2: Sample Pod containing 17.5  $\mu$ L of blood, [tassoinc.com](http://tassoinc.com)

## CONCLUSION

- An accurate/precise method has been developed to extract and analyze BPC-157 from human blood collected on a Tasso-M20 device
- A dynamic range from 1.00-2000 ng/mL was developed