Targeted Proteomics Method for Precise Quantification of Hepcidin in Human Plasma

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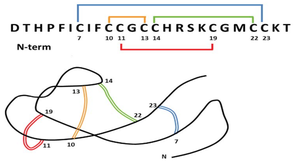
Overview

- Hepcidin, a cysteine-rich peptide hormone, plays a central role in iron metabolism regulation.
- It is considered as a biomarker and a potential target to facilitate the diagnosis and treatment of iron disorder-related diseases, including cancers.
- We have developed a simple reliable targeted LC-SRM method for sensitive quantification of Hepcidin in complex biological matrices.



Introduction

- Hepcidin, a 25-amino-acid protein with eight cysteine residues and four disulfide bonds between the two anti-parallel strands.
- Hepcidin is well known as iron-regulatory hormone, considered as a biomarker and a potential target to facilitate the diagnosis and treatment of iron disorder related diseases including cancers.
- It remains challenging to apply mass spectrometry (MS)-based Hepcidin assays for precise quantification due to low fragmentation efficiency and the difficulty in synthesis of intact Hepcidin standard.
- To address these two challenges we recently developed a reliable MS method by replacement of intact Hepcidin standards with fully alkylated Hepcidin standards.

Sample Prep. Workflow

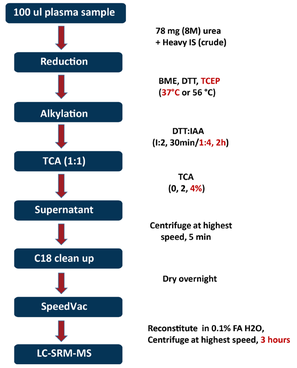


Figure. 1. Sample prep workflow for precise targeted quantification of Hepcidin in plasma and media

SRM Transitions

Optimized SRM method and selected target compounds.

Compound name	Precursor Ion[2+]	Product ion	Collision energy	Fragment
DTHFPICIFCCGCCHRSKCGMCCKT, m/z 3251.08	651.259	501.209	22	$[b4]^{+}$
	651.259	**756.943**	**12**	$[y17]^{3+}$
	651.259	**794.637**	**11**	$[y18]^{3+}$
	651.259	847.981	11	$[y19]^{3+}$
DTHFPICIFCCGCCHRSKCGMCCKT, m/z 3259.08	652.862	501.209	22	$[b4]^{+}$
	652.862	**759.614**	**12**	$[y17]^{3+}$
	652.862	**797.309**	**11**	$[y18]^{3+}$
	652.862	850.652	11	$[y19]^{3+}$

C+ C[+57.0], K+ $K[^{13}C_6,{}^{15}N_2]$; **Ions chosen as quantifiers in bold**, the others were used as qualifiers.

Table 1. The optimal transitions and collision energies for both light and heavy-labeled alkylated Hepcidin.

LC-SRM Analysis

MS: TSQ Vantage triple quadrupole (Thermo Scientific),
- Scan width: m/z 0.002
- Dwell time: 75 ms

LC: nanoACQUITY UPLC system (Waters)
- Mobile phase A: 0.1% formic acid in water
- Mobile phase B: and 0.1% formic acid in 90% acetonitrile.
- Gradient time: 75 min, 5-20 % B in 35 min, 20-25 % B in 10 min, 25-38 % B in 8 min, 38-95 % B in 1 min, and 95 % for 6 min.
- Flow rate: of 400 nL/min

MS/MS Fragments

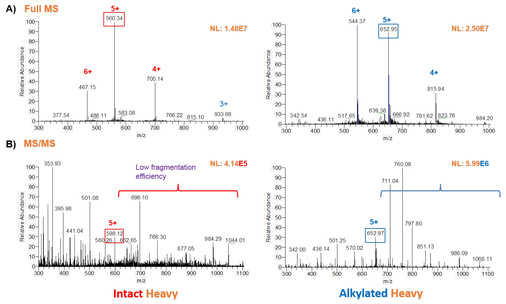


Figure. 2. (A) MS spectra of intact and alkylated isotopic Hepcidin-25. (B) Representative MS/MS spectra that shows low fragmentation of the intact Hepcidin if compared to the alkylated form.

Calibration Curve

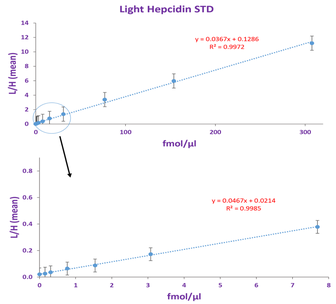


Figure 3. Calibration curve for quantifying Hepcidin. High-purity alkylated light Hepcidin with a concentration range from 0.5-1000 ng/mL was spiked into the control plasma with negligible endogenous Hepcidin. The inset plot shows details of the low concentration points.

LOD/LOQ in Plasma

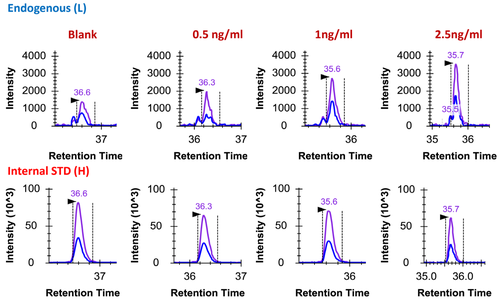


Figure 4. Extracted ion chromatograms (XIC) of transitions monitored for alkylated endogenous and heavy Hepcidin in control serum. Limits of detection (LOD) and quantification (LOQ) were determined to be ≤0.5 ng/mL and 1 ng/mL, respectively; equal to or better than current MS-based Hepcidin assays.

Human Plasma

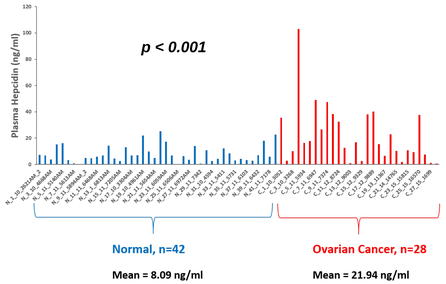


Figure 5. Endogenous Hepcidin levels in 70 clinical plasma samples (42 normal and 28 ovarian cancer patients. Measured Hepcidin concentration ranged from 0.18 to 102 ng/mL across normal and cancer samples with our assay.

HepG2 Media

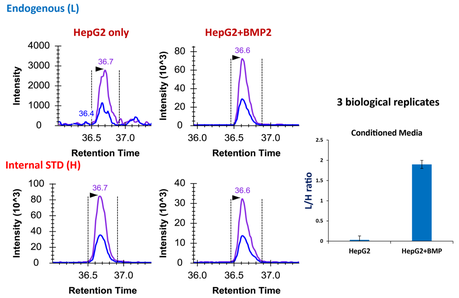


Figure 6. XICs of transitions monitored for alkylated endogenous and heavy Hepcidin in hepatocytes cell media (HepG2) in the presence and absence of BMP6 (10 ng/ml). The right panel shows L/H Hepcidin ratios for both HepG2 control and with BMP6 (triplicate for each condition).

Conclusions

- We have developed a simple reliable LC-SRM method for sensitive quantification of Hepcidin.
- Our results demonstrate that the newly developed Hepcidin detection method has similar or better sensitivity and quantification accuracy than current MS-based Hepcidin assays, without the challenges in synthesis and cost presented by the need for intact Hepcidin standards.

References

1. Pharmacol Ther. 2015 Feb;146:35-52.
2. Acta Haematol. 2009 Nov; 122(2-3): 78–86.

Acknowledgements

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Career Opportunities: For potential openings in Integrative Omics Group at PNNL please visit: http://omics.pnl.gov/careers

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