

Analysis of Hepcidin from Urine Using Sample Purification On Chip (SPOC) - MALDI

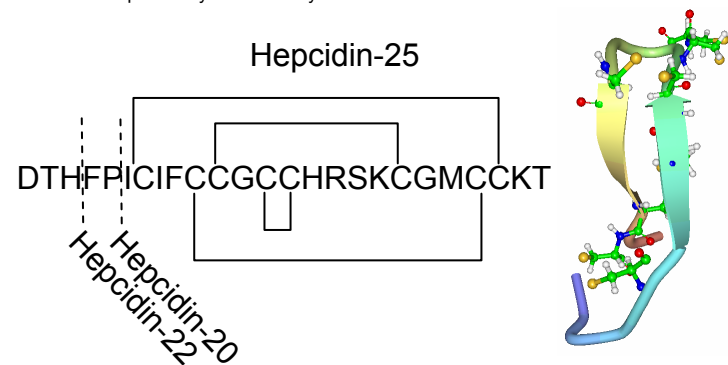


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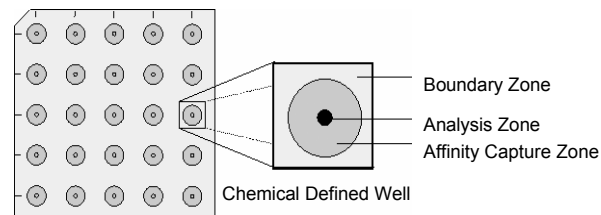
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Introduction

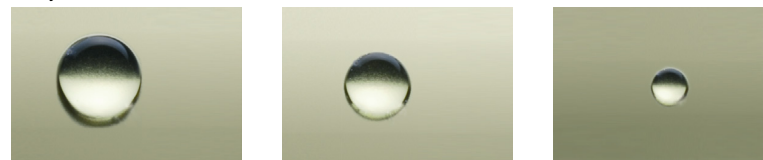
Hepcidin is a cysteine-rich peptide hormone critical in iron metabolism. Quantifying excreted hepcidin in urine is of interest because of its relationship to conditions such as anemia, inflammation and hypoxia. In high-throughput screening efforts using tandem mass spectrometry (MS/MS), challenges include reliable and reproducible isolation and sequencing of potential biomarkers and discrimination of important and meaningful biomarkers from ambiguous pattern artifacts. Our approach demonstrates recent optimization of the design of and protocols for a MALDI-MS chip that applies Sample Preparation On a Chip (SPOC) technology for the profiling of urine samples. This technique facilitates efficient, simple, and reproducible isolation of proteins/peptides and also offers the possibility to effectively concentrate low-abundance molecules.



SPOC - chip based sample preparation:



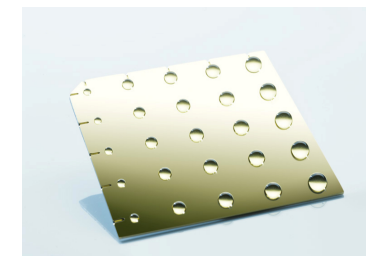
On Mass-Spec-Focus Chips (QIAGEN), samples are applied to "chemically defined wells". A hydrophobic *Boundary Zone* confines the drop and prevents it from spreading. The functionalized *Affinity Capture Zone* specifically retains the target analytes / peptides allowing contaminants to be washed away. After washing, a volume of eluent solution is added to the well. The analyte is resolubilized and the analyte and matrix are co-focused into the *Analysis Zone*, giving a highly concentrated and homogenous analyte:matrix spot ready for MALDI analysis.



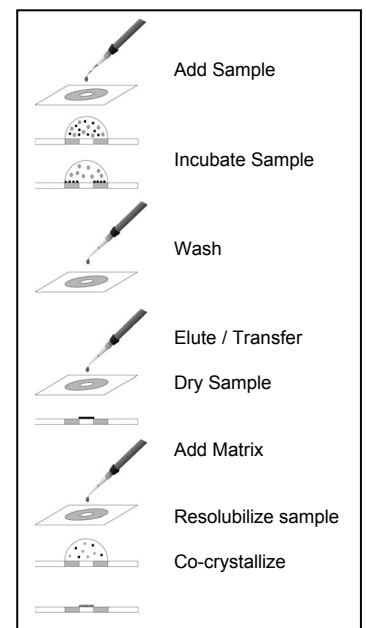
On-chip sample concentration on Mass-Spec-Focus Chips.

Method

- Incubate undiluted urine samples (15µl) on RP biochip – 30 minutes
- Remove sample and wash with excess of HPLC grade water (3x 20ul) – 5 min each, dry with nitrogen stream.
- Add 2 µl of matrix solution (0.1 mg/ml HCCA in 9:1 ACN/0.1% TFA [aq])

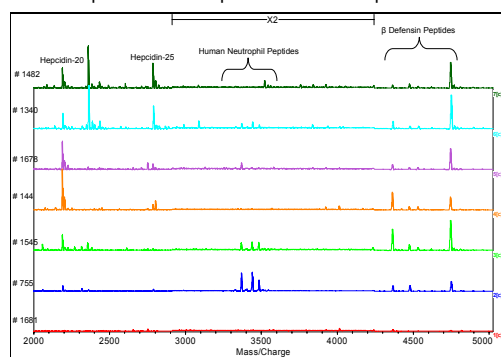


Mass-Spec-Focus Chip



MALDI spectra of urine on Mass-Spec-Focus Chips- RP

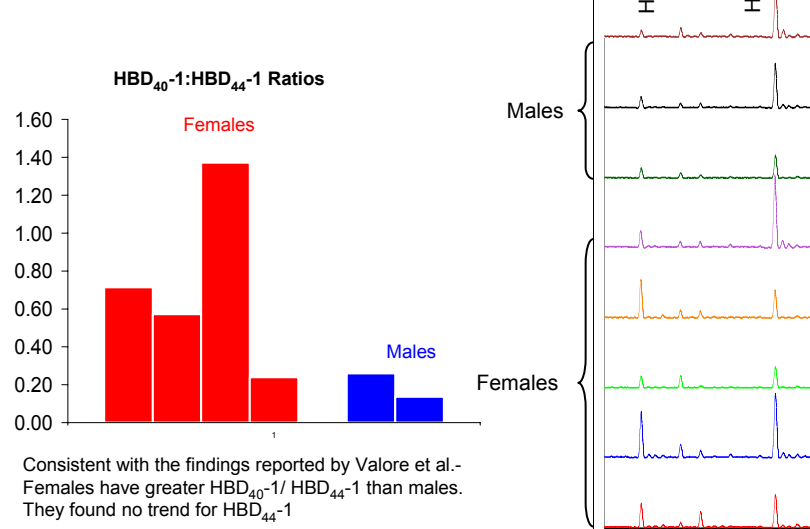
MALDI spectra of 7 independent urine samples



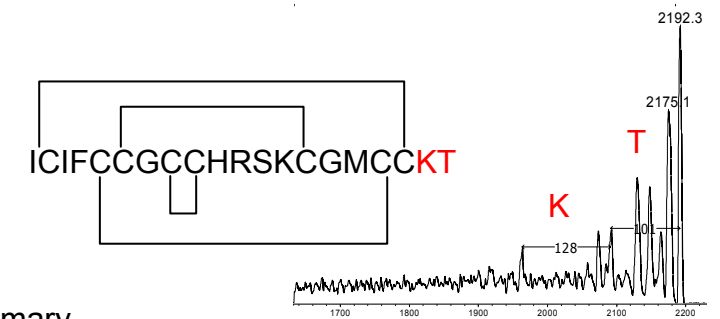
HNP = Human Neutrophil Peptide (alpha defensins)
HBD-1 = Human Beta Defensin-1

* = based on TagIdent mass search of Swiss-Prot and TrEMBL databases with $\pm .05\%$ Tolerance
† = corroborated by previous SELDI urine cleanup studies – Kemna et al. (Clin Chem 2007. 53: 620-628) , Evans-Nguyen et al. ASMS 2006 poster
^ = corroborated by SELDI alkylation experiments previously done by author
= based on LC-MS data in literature for urine samples- Valore et al. (J. Clin. Invest. 1998. 101: 1633-1642)

Human Antimicrobial Peptides – Gender Distribution



CID of Urine 144 Hepcidin 20 Peak (AXIMA-TOF²)



Summary

- The Mass-Spec-Focus Chip provides a singular platform for performing Sample Preparation on Chip (SPOC) with urine.
- In addition to Hepcidin, several antimicrobial peptides were also purified and detected by the SPOC procedure. These peptides also have been reported using LC-MS (Valore et al.)
- The CID fragmentation successfully demonstrated the expected MS/MS spectra taking into account the 4 disulfide bonds.
- Future work will focus on sample pretreatment strategies and sample-processing protocols, as well as quantification and identification of the different peaks by MALDI.