

OW2 Quantitative assay of urinary hepcidin- a key regulator of iron metabolism

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Validated method for quantification of endogenous urinary hepcidin by MALDI-O-TOF

Hepcidin, a peptide hormone synthesized in the liver, is the principal regulatory hormone in iron homeostasis[1]. Hepcidin is induced by inflammation, iron overload and anaemia of inflammation, while low hepcidin levels are linked to hypoxia, anaemia and iron deficiency[1,2]. It is also involved in the pathogenesis of hereditary hemochromatosis. An assay is essential for the diagnosis and monitoring of the disorder.

A simple SPE clean-up followed by MALDI-O-TOF-MS was developed for the identification and quantification of endogenous urinary hepcidin[3]. Standard addition of hepcidin into extracted urine with internal standard was used in the quantification process. Hepcidin was normalised with urinary creatinine which was analysed by HPLC.

Greater than 98% extraction recovery of hepcidin was achieved with minimal oxidation of the methionine amino acid. Spot-to-spot variation (n=15) of hepcidin was under 3.5%. Intra- (n=5) and inter-day (n=3) precision assay of less than 9.5% RSD was achieved with less than 0.5% variation between the intra-day assay data.

A validated method has been developed for endogenous urinary hepcidin for the diagnosis and monitoring of iron-related disorders.

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[2] E.H.J.M. Kemna, H. Tjalsma, H.L. Willems, D.W. Swinkels. *Haematologica*. 2008, 93: 90-97.

[3] M.C.L. Gay. R.D. Trengove. Quantitative assay of urinary hepcidin using MALDI-O-TOF-MS. Unpublished manuscript.