P007 The use of solid phase extraction cartridges as an alternative to ultrafiltration for the measurement of non transferrin bound iron in plasma

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This study examined the feasibility of using solid phase extraction [SPE] cartridges as a form of size exclusion chromatography to enable the measurement of non transferrin-bound iron [NTBI] in plasma without the need for ultrafiltration. The principle being that plasma is reacted with a chromophore which selectively binds NTBI. The incubated plasma is loaded directly onto the SPE cartridge. The NTBI-chromophore complex binds to the SPE cartridge allowing the protein to wash through with appropriate volumes of water. The iron-chromophore complex is then eluted and quantified by HPLC. A number of SPE cartridges and chromophores were investigated. The most successful cartridge with regard to the desired characteristics was chromabond C18ec 50mg-1ml. The substance which appeared closest to fulfilling the criteria for the ideal chromophore (outlined above) was the iron chelator deferoxamine [DFX] which, in the absence of other chelators such as nitrilotriacetic acid, cannot remove significant amounts of iron bound to transferrin. The iron-bound form of DFX ferrioxamine is readily quantified by HPLC with UV detection. The levels of plasma NTBI measured using DFX and just SPE separation or ultrafiltration showed remarkable similarity. The chromophore 3-(2-pyridyl)-5,6 di (2-furyl)-1,2,4 triazinesulphonic acid [PFS] selectively binds Fe^{2+}, does not remove iron from protein, is easily separated from protein by SPE extraction and is easily quantified by HPLC. This paper explains the development of these methods and their use in measuring plasma iron.