

Table 1B: Summary of methods and technologies employed for the analysis of the standard urine samples. UF: ultrafiltration; MWCO: Molecular weight cut-off; N/A: not applicable; H: Hannover; G: Glasgow.

Technique	Sample preparation	Separation	Digestion	MS	Identification			Quantification
					Engine	Modifications	Accuracy	
2DE	UF (3000MWCO) TCA/NLS precipitation	IEF 3-10, 11%PAGE	trypsin	MALDI TOF TOF	Mascot 2.2.01	Fixed modification: carbamidomethylation (Cys) ; Variable: Oxidation (Met)	25ppm (MS)	N/A
GeLCMS	UF(5000MWCO)	4-12% Bis-Tris PAGE and Ultimate 3000 coupled to MS	oxidation trypsin	LTQ-FT Ultra	Mascot 2.2.03	Variable modifications: N-Ac- protein, methionine oxidation and dioxidation, cystein trioxidation and proline hydroxylation	10 ppm (MS), 0.8 Da (MS/ MS)	N/A
CE-MS	UF (20000MWCO), desalting (PD-10), lyophilization	P/ACE MDQ CE coupled to MS	none	microTOF MS (H) or microTOF-Q MS/MS (G)	Internet version, OMSSA 2.1.4	Variable modifications: oxidation of methionine, lysine, and proline	0.5 Da (MS), 0.7 Da (MS/MS)	Internal standards and spiked isotope labeled peptides
LC-MS and MS/MS	UF (20000MWCO), desalting (PD-10), lyophilization	Ultimate3000 coupled to MS	none	LTQ-Orbitrap	Mascot 2.2.03	Variable modifications: oxidation of methionine, lysine and proline	10 ppm (MS), 0.8 Da (MS/ MS)	Internal standards

