ELECTRONIC SUPPLEMENTARY INFORMATION to

Detection of Proteins by Hyphenated Techniques with Endogenous Metal Tags and Metal Chemical Labelling

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Table 1. Analytical methods for the species-selective analysis of selenoproteins/peptides by hyphenated techniques with element-selective detection.

Analyte	Sample type	Separation technique	Column	Eluent	Detector	Detection limit	Ref.
SeCys GPx SelP SelAlb	Human serum	Affinity HPLC	Hitrap Heparin-Sepharose (HEP) (5 cm×1.0 mm id) Hitrap Blue-Sepharose (BLUE) (5 cm×1.0 mm id)	(A): 0.05 M ammonium acetate, pH~7 (B): 1.5 M ammonium acetate, pH~7	ICP - sector field-MS in high resolution mode	2.5 ng mL^{-1} for SeCys	86
GPx SelP SelAlb	Human serum reference material (BCR-637)	Affinity HPLC	Hitrap Heparin–Sepharose and Hitrap blue-Sepharose (1 mL each)	(A): 0.02 M Tris-HCl buffer, pH 7.4 (B): A + 1.4 M ammonium acetate, pH 7.4	ICP-quadrupole-MS	0.4 ng mL^{-1} (GPx) 2.0 ng mL^{-1} (SelP) 1.0 ng mL^{-1} (SelAlb)	88
SelP isoforms	Human serum reference material (SRM 1950)	SDS-PAGE Nano reversed phase HPLC	Nano cHiPLC column (15 cm×75 μm, ChromXP C18-CL 3 μm)	*SDS*: NuPAGE® MES SDS Running Buffer *RPC*: (A): water/0.1% formic acid (B): acetonitrile/0,1% formic acid Gradient: from 2-25.8% B in 75 min and from 25.8- 90% B in 10 min	ICP-MS ESI-linear triple quadrupole-MS	NR	89
GPx SelP SelAlb Two unknown selenospecies	Human plasma	Anion exchange HPLC	ProPac SAX-10 (250 mm×2 mm id, 10 μm particles)	(A): 10 mM Tris-acetic acid buffer, pH 8.0 (B): A + 500 mM ammonium acetate, pH 8.0 Gradient: 100% A for 3 min; 0-40% B in 7 min; 40-100% B in 6 min;	ICP-dynamic reaction cell-quadrupole-MS	0.59 pmol mL^{-1} (SelP) 1.7 pmol mL^{-1} (GPx)	82

Analyte	Sample type	Separation technique	Column	Eluent	Detector	Detection limit	Ref.
GPx SelP SelAlb	Human plasma standard reference material (SRM 1950)	Affinity HPLC Nano-reversed phase HPLC	*AFC:* PEEK columns (50 mm×4 mm id) packed with heparin-Sepharose (HEP) or blue-Sepharose (BLUE) *RPC:* ChromXP C_{18}-CL (15 cm×75 µm id, 3 µm particles, 120 Å;)	100% B for 19 min *AFC*: (A): 50 mM ammonium acetate (B): 1.5 M ammonium acetate Gradient: from 0-100% B in 6 min *RPC*: (A): water/0.1% formic acid (B): acetonitrile/0.1% formic acid Gradient: from 2-25.8% B in 75 min; 25.8-90% B in 10 min *SDS*: NuPAGE® MES SDS Running Buffer	LA-ICP MS ESI-LTQ ion trap-MS	NR	[90]
SelP GPx3	Human plasma candidate standard reference material (SRM 1950)	SDS-PAGE Electroblotting onto PVDF membrane Nano reversed phase HPLC	*RPC*: BioBasic C18 IntegraFrit column (100 cm×10 µm id)	*RPC*: (A): water/0.1% formic acid (B): acetonitrile/0.1% formic acid Gradient: from 2–25% B in 75 min; 25–85% B in 10 min; 90% B for 9 min	LA-ICP-MS ESI-LTQ ion trap-MS	8 ng of GPx1 for electroblotting-LA-ICP MS	[91]
GPx	Selenium-yeast candidate reference material	SDS-PAGE	-	-	Electrothermal vaporization-ICP MS	40 pg Se per band	[92]
Selenomethionine	Yeast	Gas	DB-5MS	Carrier gas: helium	ICP-sector field-	1.0 µg g^{-1}	[93]

Analyte	Sample type	Separation technique	Column	Eluent	Detector	Detection limit	Ref.
	extracts	chromatography			MS		
Selenomethionine Selenocysteine	Human serum	Size exclusion HPLC Capillary reversed phase HPLC	*SEC*: HiLoad 26/60 Superdex 30 Prep *RPC*: Hypersil C_{18} BDS (15 cm×300 μm id, 3 μm particles)	*SEC:* 10 mM ammonium acetate, pH 9.5 *RPC:* (A): 5% acetonitrile/0.1% trifluoroacetic acid (B): 95% acetontrile/0.1% trifluoroacetic acid Gradient:from 10-60% B in 15 min; 60% B for 5 min	ICP-octapole reaction cell-MS	0.5 ng of Se/g of serum	94
SelP	Sub-μL samples of human plasma	Size exclusion HPLC Affinity HPLC	*SEC*: Asahipak GS-520 (300 mm×7.6 mm id) *AFC*: AFpak AHR-894 (150 m×4.6 mm id)	*SEC*: 50 mM Tris–HNO_3, pH 7.4 *AFC*: 20 mM Tris–HNO_3 + 1.4 M ammonium acetate, pH 7.4	Low flow ICP-MS	0.15 ng mL^{-1} (Nebulizer PFA100) 0.72 ng mL^{-1} (Nebulizer PFA 20) 0.49 ng mL^{-1} (Nebulizer CEI 100)	87
Mixture with more than 30 selenopeptides	Selenized yeast extract	Capillary reversed phase HPLC	Hypersil C_{18} BDS (15 cm×300 μm id, 3 μm particles)	(A): water/0.1% trifluoroacetic acid (B): methanol/0.1% trifluoroacetic acid Gradient: from 10-25% B in 5 min; 25-32% B in 15 min; 32-37% B in 5 min; 37% B for 10 min; 37-42% B in 10 min, 42-100% B in 5 min.	ICP-collision cell-MS	150 fg (^{80}Se) 200 fg (^{82}Se)	55
GPx	Human plasma	Size exclusion	*SEC*:	*SDS-PAGE*:	ICP-MS	NR	95

Analyte	Sample type	Separation technique	Column	Eluent	Detector	Detection limit	Ref.
SelP SelAlb		HPLC Capillary reversed phase HPLC SDS-PAGE	Superdex G-75 HR 10/30 *RPC*: C_{18} PepMap (15 cm×300 μm id, 3 μm particles)	25 mM Tris buffer + 3.5 mM SDS + 192 mM L-glycine *SEC:* 50 mM ammonium acetate, pH 7 *RPC*: (A): water/0.1% trifluoroacetic acid (B): methanol/0.1% trifluoroacetic acid Gradient: 5% B for 2 min; 5–60% B in 48 min; 60–90% B in 5 min; 90% B for 3 min; 90–5% B in 7 min			
GPx SelP SelAlb	Human serum samples and reference materials	Double affinity followed by size exclusion HPLC	*AFC*: Heparin Sepharose (HEP-HP) and Blue Sepharose (BLUE-HP) (5 cm×4.6 mm id) *SEC*: Shodex Asahipak GS-520 HQ (300 mm×7.6 mm id, 7 μm particles)	(Binding buffer): 0.05 M ammonium acetate, pH~7 (Elution buffer): 1.5 M ammonium acetate, pH~7	ICP-MS	NR	[80]
Selenomethionyl calmodulin	Protein obtained by heterologous expression in *Escherichia coli*	Reversed phase nano HPLC	C_{18} PepMap (15 cm×75 mm id, 3 μm particles, 100Å)	(A): 0.1% trifluoroacetic acid in water (B): 0.1% trifluoroacetic in acetonitrile Gradient: 5% B isocratic in 3 min, 5–25% B in 5 min, 25–37% B in 9 min,	ICP-octapole reaction cell-MS ESI-quadrupole/time of flight-MS	40 fg (^{80}Se)	[96]

Analyte	Sample type	Separation technique	Column	Eluent	Detector	Detection limit	Ref.
				37–45% B in 8 min; 45% B isocratic in 14 min, 45–90% B in 1 min, 90% B isocratic in 3 min			
SelP	Human and mouse plasma	Affinity HPLC Size exclusion HPLC	*AFC*: Heparin AHR-894 (50 mm×8.0 mm id) *SEC*: Asahipak GS-520 7G (500 mm×7.5 mm id) + guard column Asahipak GS-2G 7B (50 mm × 7.5 mm id)	(Binding buffer): 50 mM Tris–HNO_3 buffer, pH 7.4 (Elution buffer): A + 1.4 M ammonium acetate.	ICP-MS	NR	[97]
GPx SelP SelAlb	Human serum	Anion exchange HPLC Affinity HPLC	*AEC:* Mono Q 5/5 FPLC analytical column (50×5 mm id) *AFC:* Hitrap Heparin–Sepharose and Hitrap blue-Sepharose (1 mL each)	*AEC*: (A): 0.05 M Tris-HCl buffer, pH 7.4 (B): A + 0.5 M ammonium acetate, pH 7.4 Gradient: 100% A for 2 min; 0-15% B in 6 min; 15- 45% B in 6 min; 45-100% B in 5 min; 100% B for 21 min *AFC:* (A): 0.02 M Tris-HCl buffer, pH 7.4 (B): A + 1.4 M ammonium acetate, pH 7.4	ICP-octapole reaction cell-MS	NR	[98]
GPx Formate	Bacterial cultures of	SDS-PAGE Size exclusion	*SEC:* Superdex 200 HR 10/30	*SDS-PAGE*: 25 mM Tris buffer + 3.5	LA-ICP-MS ICP-MS	Low-femtomolar levels	[99]

Analyte	Sample type	Separation technique	Column	Eluent	Detector	Detection limit	Ref.
dehydrogenase selenoprotein	*Desulfococcus multivorans* and *Escherichia coli*	HPLC	column	mM SDS + 192 mM L-glycine *SEC*: 10 mM ammonium acetate, pH 7.5			
GPx3 SelP SelAlb	Human serum form patients with colorectal cancer	Anion exchange HPLC Double affinity HPLC	*AFC*: Hitrap Heparin–Sepharose and Hitrap blue-Sepharose (5 cm×4.6 mm id) *AEC*: Styrene divinylbenzene with quaternary ammonium functional groups (5 cm×4.6 mm id, 45-150 μm particles)	(Binding buffer): 0.02 M Tris-HCl buffer, pH 7.4 (Elution buffer): A + 1.4 M ammonium acetate, pH 7.4	ICP-quadrupole-MS	NR	[100]
Selenomethionine	Selenium-enriched yeast	Gas chromatography	DB-5MS (30 m×0.25 mm id×0.25 μm d_f)	Carrier gas: helium	MS	NR	[101]
Se-rich glutenins	Wheat	Isoelectric focusing separation 1-D SDS-PAGE IEF/SDS-PAGE 2D gel electrophoresis Reversed phase HPLC	*RPC*: C_{18} Zorbax 300SB (100 mm × 0.3 mm id, 3.5 μm particles)	*RPC*: (A): 0.1% formic acid in water (B): 0.1% formic acid in acetonitrile Gradient: 2% B for 2 min, from 2−5% B in 3 min, 5−25% B in 30 min, 25−40% B in 5 min, 40−97% B in 5 min; 97% B for 5 min	LA-ICP-MS ICP-MS ESI-linear triple quadrupole/Orbitrap-MS	Attomolar levels	[102]

Table 2. Analytical methods for the species-selective analysis of iron-containing proteins by hyphenated techniques with element-selective detection.

Analyte	Sample type	Separation technique	Column	Eluent	Detector	Detection limits	Ref.
Myoglobin Ferritin	Raw and cooked beef steak	Size exclusion HPLC	Progel TSK Gel G2000SW$_{XL}$ (300 mm×7.6 mm id,10 μm particles, 125 Å) + guard column Progel TSK Gel G2000SW$_{XL}$ (5×7.6 mm id, 10 μm particles, 125 Å)	0.1 M Tris(hydroxymethyl)aminomethane hydrochloride pH 7.2	ICP-double-focusing sector field-MS	0.0024 μg as iron	106
Transferrin	Serum samples from human and harbour seals	Anion exchange HPLC	PorosHQ (100 mm×2.1 mm id, 10 μm particles)	(A): 20 mM Bis-Tris, pH 6.5, 10 μg L^{-1} Ge (B): 20 mM Bis-Tris, 500 mM ammonium acetate, pH 6.5, 10 μg L^{-1} Ge, Cs	ICP-octapole reaction cell-MS	123 μg as transferrin	107
Transferrin isoforms	Human serum from healthy individuals and alcoholic patients	Anion exchange HPLC	Mono Q HR 5/5 (50 mm×5 mm id)	(A): 25 mM Tris-HAc buffer, pH 6.5 (B): A + 250 mM ammonium acetate Gradient from 0%-75% B in 45 min	ICP-octapole reaction cell-MS ESI-quadrupole/time of flight-MS	15 ng mL^{-1} Fe in a low abundance form of transferrin	69
Nine transferrin glycoforms	Blood samples of harbour seals	Anion exchange HPLC	PorosHQ (100 mm×2.1 mm id, 10 μm particles)	(A): 20 mM Bis-Tris, pH 6.5, 10 μg L^{-1} Ge (B): 20 mM Bis-Tris, 500 mM Ammonium acetate, pH 6.5, 10 μg L^{-1} Ge, Cs	ICP-octapole reaction cell-MS	55.2 μg mL^{-1} as transferrin	108
Five transferrin isoforms	Human serum	Capillary zone electrophoresis or anion exchange HPLC	*AEC:* Pharmacia, Mono Q 5/5 HR *CE capillary*: 50 μm i.d×75 cm	*AEC*: (A): 25 mM Tris-HCl buffer, pH 6 (B): A + 250 mM ammonium acetate	UV ICP-octapole reaction cell-MS	0.27-0.37 μmol L^{-1} CZE-UV 14-27 μmol L^{-1} CZE-ICP MS	109

Analyte	Sample type	Separation technique	Column	Eluent	Detector	Detection limits	Ref.
				Gradient: from 0-75% B in 45 min *CE:* 15 mM borate buffer, pH 8.5 + 3 mM diaminobutane		0.05-0.15 μmol L^{-1} HPLC-UV 0.02-0.04 μmol L^{-1} HPLC-ICP MS	
Myoglobin Holo-transferrin	Proteins standard solutions	Size exclusion HPLC Reversed phase HPLC	*SEC:* Sephadex G-50 Fine (370 cm×16 mm id) *RPC:* Polyester C-CP fibers in stainless steel column (150 mm×4.6 mm id)	*SEC:* 100% Nanopure water at pH 5 *RPC:* Nanopure water containing/0.1% trifluoroacetic acid, pH 5	ICP-OES Particle beam/hollow cathode-OES	0.9 ng mL^{-1} (for ICP OES) and 41.9 ng mL^{-1} (for PB/HC-OES) as Fe	[110]
β_2-transferrin	Cerebrospinal fluid	Anion exchange HPLC	Mono Q HR 5/5 (50 mm×5 mm id)	(A): 25 mM Tris-HAc buffer, pH 6.5 (B): A + 250 mM ammonium acetate Gradient from 0-75% B in 45 min	ICP-octapole reaction cell-MS ESI-quadrupole/time of flight-MS	NR	[111]
Cytochrome C Haemoglobin Transferrin Ferritin	Proteins standard solutions	SDS-PAGE Anodal native PAGE Cathodal native PAGE	-	*SDS-PAGE and Anodal PAGE:* Separating gel buffer: 27.30 g of Tris in 80 mL, pH 8.8 Stacking gel buffer: 6 g of Tris in 60 mL of water, pH is 6.8 Electrode running buffer: 30.3 g of Tris and 144.0 g of glycine in 1 L of water, pH 8.3. Elution buffer: 0.02 M Tris pH 8.3. *Cathodal PAGE:* Separating gel buffer: 5 g of Tris in 1 L of distilled water,	ICP-MS	Ranging from 0.17 to 0.76 ng as Fe	[112]

Analyte	Sample type	Separation technique	Column	Eluent	Detector	Detection limits	Ref.
Ferritin	Edible plant seeds	Anion exchange HPLC	Diethylaminoethyl Sepharose (10 cm×0.7 cm id)	pH 5 Anode buffer: 26.24 g of 6-aminocaproic acid in 1 L of water, pH 5.3 Cathode buffer: the same buffer as separating gel. Elution buffer: 0.02 M Tris with HCl at a pH 7. (A): 20 mM Tris + 1 mM EDTA, pH 7.4 (B): 20 mM Tris + 1 mM EDTA + 100 mM NaCl, pH 7.4 (C): 20 mM Tris + 300 mM NaCl + 1 mM EDTA, pH 7.4	Sector field-MS	0.06 μg of ferritin	113
Glycated and non-glycated haemoglobin	Human blood from healthy individuals and diabetic patients	Cation exchange HPLC	Cation Exchange Mono S 4.6/100 PE (100 mm×10 mm id, 10 μm particles)	(A): 50 mM ammonium malonate + 0.2 g L^{-1} NaN_3, pH 5.7 (B): A + 250 mM ammonium acetate, pH 5.7 Gradient: from 0-60% B in 6 min; 60-75% B in 4 min; 75-100% B in 5 min	ICP-octapole reaction cell-MS ESI-quadrupole/time of flight-MS	0.97±0.02 μg mL^{-1} of glycated haemoglobin	114

Table 3. Analytical methods for the species-selective analysis of copper-containing proteins by hyphenated techniques with element-selective detection.

Analyte	Sample type	Separation technique	Column	Eluent	Detector	Detection limits	Ref.
Superoxide dismutase	Tissue samples from bovine liver	Non denaturing 1-D PAGE	-	Running buffer: 25 mM Tris–base + 192 mM glycine solution	LA-ICP-MS	0.5 μg of protein	68
Four native and recombinant copper proteins	Cell extracts from *Escherichia. coli* and *Synechocystis*	Capillary reversed phase HPLC Size exclusion HPLC Anion exchange HPLC	*RPC*: Poroshell SB C_8 (0.5 mm×75 mm) *SEC*: Sephadex G25 superfine (350mm ×2.0 mm) *AEC:* TSK-DEAE-NPR column (particle diameter 2.5 μm)	*RPC:* (A): water/5% acetic acid (B): acetonitrile/5% acetic acid Gradient: from 10-50% B in 1 min; 50% B for 5 min *SEC*: 150 mM NaCl + 20 mM Tris-HCl, pH 7.4 *AE:* (A): 20 mM Tris, pH 8.5 (B): 1 M NaCl + 20 mM Tris, pH 8.5 Gradient: from 0-50% B in 10 min	ICP-dynamic reaction cell-MS ESI-time of flight-MS	Ranging from 650 to 800 fmol of plastocyanin	117
Ceruloplasmin	Human serum from four different diseases and a set of normal controls	Size exclusion HPLC Reversed phase HPLC	*SEC:* Silica TSKGel SW3000 (30 mm×4.6 mm id, 4 μm particles) *RPC:* Zorbax 300SB-C_{18} (75 μm×150 mm, 3.5 μm particles)	*SEC:* 0.1M Tris, pH 7 *RPC:* (A): water/0.1% formic acid (B): acetonitrile/0.1% formic acid Gradient: from 0-95% B in 60 min	ICP-octapole reaction cell-MS ESI-ion trap-MS	0.01 mg ml^{-1} of ceruloplasmin	118

Transcuprein Ceruloplasmin	Human plasma from healthy subjects and an untreated Wilson disease patient	Size exclusion HPLC	BioSep-SEC-S 2000 and Biosep-SEC-S 3000 (300 mm×7.8 mm both)	200 mM NH_4NO_3	ICP-dynamic reaction cell-MS	Ranging from 4.8 to 27 nmol L^{-1} based on the method used (flow injection or LC)	[119]
Albumin-copper Ceruloplasmin	Human serum	Size exclusion HPLC	Superose 12HR	0.1 M Tris-HCl buffer + 2.5 mM $CaCl_2$, pH 7.4	ICP-quadrupole-MS	1 ng ml^{-1} as Cu	[120]

Table 4. Analytical methods for the species-selective analysis of metallothioneins by hyphenated techniques with element-selective detection.

Analyte	Sample type	Separation technique	Column	Eluent	Detector	Detection limits	Ref.
Cd MTs	Standard solutions of rabbit liver Cd-MTs	Capillary electrophoresis	Fused-silica uncoated capillaries (75 μm i.d., 363 μm o.d.)	70 mM TRIS buffer pH 7.4 + 5% methanol	Volatile species generation – ICP-quadrupole-MS	Ranging from 119 to 259 ng mL^{-1} as Cd	130
Zn, Cu and Cd MTs isoforms	Rat liver tissue	Capillary zone electrophoresis	Uncoated fused silica capillaries (75 μm i.d., 70 and 100 cm long)	(A): 20 mM Tris–HNO_3, pH 7.4 (B): 25 mM ammonium acetate, pH 6.8	ICP-sector-field double-focusing-MS ESI-MS	80 μg mL^{-1} of MT	131
Up to five Zn, Cu and Cd MT isoforms	Cytosolic extracts of carp *Carassius auratus gibelio*	Size-exclusion HPLC Anion-exchange HPLC	*SEC:* TSK gel G 3000 PWxL (30 cm×7.8 mm id, 6 μm particles) *AEC:* Protein-Pak DEAE-5PW (7.5mm×75mm id).	*SEC*: 30 mM Tris–HCl, pH 7.4 *AE*: (A): 2 mM Tris–HCl, pH 7.4 (B): 200 mM Tris–HCl, pH 7.4 Gradient: from 0-15% B in 10 min; 15-20% B in 5 min; 20-50% B in 5 min; 50% B for 5 min	ICP-time-of flight-MS	NR	132
Zn, Pb, Cu and Cd MTs isoforms	Hepatic cytosols of Cd exposed carp *Cyprinus carpio*	Reversed phase HPLC	Spherisorb ODS 2 (250 mm×4.6 mm id, 5 μm particles)	(A): 1% methanol/30 mM ammonium acetate in, pH 7.4 (B): methanol/30 mM ammonium acetate in, pH 7.4 Gradient: from 0-30% B in 15 min; 30% B for 10 min	ICP-time-of flight-MS ESI-time of flight-MS	NR	133
Zn and Cu MTs	Human peripheral blood mononuclear cells	High resolution size exclusion HPLC	Waters Biosuite 450 8 μm UHR SEC (250 mm×4.6 mm id) + guard UHR SEC (35	0.1 M Tris-HCl + 0.05% NaN_3, pH 7.2	ICP MS	0.5 ng for total MTs, 0.3 pg for Zn, 0.2 pg	134

			mm×4.6 mm id			for Cu	
MT-3 isoforms	Human brain cytosols	Capillary zone electrophoresis	Fused silica capillary (length 70 cm, 75 μm id)	20 mM Tris, pH 7.4	ICP-sector field-MS UV	NR	[35]
Zn and Cd MT-1 and MT-2	Standard solutions of rabbit liver Cd and Zn MT1	Capillary zone electrophoresis	Fused-silica uncoated capillaries (length 61-79 cm, 75 μm id, 363 μm od)	70 mM Tris buffer, pH 7.4 + 5% methanol	ICP-quadrupole-MS ICP-double-focusing-MS	Ranging from 15 to 135 ng mL^{-1} as Cd	[129]
Zn, Cu and Cd MTs	Cytosolic extracts of bream *Abramis brama L.*	Capillary electrophoresis	Fused silica capillaries (length 70 cm, id 75 μm).	20 mM Tris-HNO_3, pH 7.0–7.4	ICP-octapole reaction cell-MS	Ranging from 300 ng L^{-1} (^{58}Ni) to 500 ng L^{-1} (^{66}Zn, ^{55}Mn)	[135]
Al, Ba, Cu, Fe, Mn, Sr and Zn MTs	Raft mussels (*Mytilus Galloprovincialis*) cytosols	Anion exchange HPLC	ProteinPak DEAE-5PW (75 mm×8 mm id)	75 mM Tris–HCl, pH 7.4	UV ICP-OES	81, 6, 10, 13, 30, 9 and 123 ng g^{-1} for Al, Ba, Cu, Fe, Mn, Sr and Zn	[136]
Hg, Cd, Cu and Zn MTs	White-sided dolphin (*Lagenorhynchus acutus*) liver homogenate	Hydrophilic interaction HPLC	HILIC TSK gel amide 80 (250 mm×1mm id, 5 μm particles)	(A): acetonitrile (B): 5 mM ammonium acetate, pH 5.5 Gradient: from 0-10% B in 5 min; 10-50% B in 15 min; 50% B for 5 min; 50-65% B in 2 min; 65% B for 3 min	ICP-MS ESI-hybrid linear/orbital trap-MS	NR	[137]
MTs sub isoforms	Kidney pig cell line exposed to CdS nanoparticles	Microbore reversed-phase HPLC	RP C_8 (250 mm×1 mm id, 5 μm particles)	(A): 5 mM ammonium acetate pH 6 and eluent (B): 5 mM ammonium acetate (pH 6) in 50% (v/v) acetonitrile. Gradient: from 2-20% B in 50 min	ICP-MS ESI-LTQ/Orbitrap-MS	100 fmol of protein	[138]
Zn and Cd	Mussel cytosolic	Size exclusion	*AEC:*	*AEC:*	ICP-quadrupole-	NR	[139]

MT-1 and MT-2	extracts	HPLC Anion exchange HPLC Fast liquid HPLC	Protein Pack DEAE 5PW (75 mm×7.5 mm id) *Fast liquid LC:* Mono Q HR 5/5 (50 mm×5 mm id.) *SEC:* Sephadex G-75 (100 cm×1 cm id)	(A): 2mM Tris-HCl, pH 7.4 (B) 200 mM Tris-HCl, pH 7.4 Gradient: from 0-15% B in 10 min; 15-25% B in 5 min; 50-100% B in 1 min; 100% B for 9 min *Fast liquid LC:* (A): 4 mM Tris-HCl, pH 7.4 (B): 250 mM ammonium acetate +10 mM Tris-HCl, pH 7.4 Gradient: from 0-2% B in 1.5 min; 2-3% B in 0.5 min; 3-4% B in 3.5 min, 4-10% B in 0.5 min; 10-17% B in 4 min; 17-18% B in 1 min, 18-100% B in 1 min; 100% B for 7 min *SEC:* 10 mM Tris-HCl, pH 7.4, + 5mM 2-mercaptoethanol + 0.1 mM phenylmethylsulphonyl fluoride + 25 mM NaCl	MS		
MT-1 and MT-2 isoforms	Rabbit liver cytosol and human cirrhotic livers	Size exclusion HPLC Anion exchange HPLC	*SEC:* Superdex 75 HR (300 mm×10 mm id) *AEC:* DEAE column (150 mm×10 mm id)	*SEC:* 20 mM Tris-buffer, pH 7.4 *AEC:* from 20 mM to 250 mM Tris-buffer, pH 7.4	ICP OES	NR	[140]
Cd, Zn and	Cytosolic extracts	Size exclusion	*SEC*:	*SEC:*	ICP-quadrupole-	NR	[141]

Cu MT-1 and MT-2	of eels (*Anguilla anguilla*) exposed to Cd	followed by anionic exchange fast protein HPLC	Sephadex G75 (100 cm×1 cm id) *AEC*: Mono Q HR 5/5 (5 mm×50 mm id)	10 mM Tris–HCl, pH 7.4, + 5 mM 2-mercaptoethanol + 0.1 mM phenylmethylsulphonyl fluoride + 25 mM NaCl *AEC:* (A): 4 mM Tris–HCl buffer, pH 7.4 (B): 10 mM Tris–HCl, 0.25 M ammonium acetate buffer, pH 7.4 Gradient: from 0-2% B in 1.5 min; 2-3% B in 0.5 min; 3-4% B in 3.5 min; 4-10% B in 0.5 min, 10-17% B in 4 min; 17-18% B in 1 min; 18-80% B in 2 min; 80-90% B in 2 min; 90-100% B in 1 min	MS ICP-double focusing-MS		
Cd MTs	Cd-treated and untreated rat livers	Anion exchange HPLC	Fractogel EMD DEAE-650 (S) (70 mm×10 mm id, 20–40 µm particles)	(A): 20 mM Tris–HCl, pH 7.4 (B): 200 mM Tris–HCl, pH 7.4 Gradient: from 0-60% B in 18 min	Flame AAS	2.0 µg MT-1/g liver and 1.3 µg MT-2/g liver	142
MTs isoforms	Mouse hepa cells	2-D gel filtration and anion exchange HPLC	*Gel filtration LC:* Shodex Protein KW802.5-M8E (250 mm×0.8 mm id) *AEC:* Shodex DEAE9A-M8B (50 mm×0.8 mm id)	*Gel filtration LC:* 25 mM Tris-HCl, pH 7.2 *AEC:* (A): 2 mM 1,3-propanediamine HCl, pH 9.0 (B) 100 mM 1,3-propanediamine HCl, pH 9.0	ICP-MS	NR	143

				Gradient: 0% B for 10 min; from 0-40% B in 5 min; 40-100% B in 15 min; 100% B for 10 min			
MTs isoforms	Alzheimer's disease and control brains	Size exclusion HPLC	Superdex 75 PG (600 mm× 16 mm id)	20 mM Tris-buffer, pH 7.4	UV ICP-MS	NR	[144]
Cd MTs	Cytosolic extracts of eels (*Anguilla anguilla*)	Vesicle mediated HPLC	Spherisorb C_{18} bounded silica stationary phase (250 mm×4.6 mm id, 5 µm particles) modified with didodecyldimethylammonium bromide (DDAB)	(A): 2 mM Tris-HCl + 100 mM DDAB, pH 7.4 (B): 200 mM Tris-HCl + 100 mM DDAB, pH 7.4 Gradient: from 0-50% B in 20 min; 50-100% B in 3 min; 100-99% B in 8 min	Hydride generation-ICP MS	Ranging from 8.8 and 17.1 pg as Cd	[145]
Zn, Cu and Cd MTs isoforms	Preparation of rabbit-liver MT Purified rabbit-liver MT-1	Capillary electrophoresis	Fused silica capillary (70 cm length, 75 µm id)	20 mM Tris, pH 7.4	ICP-sector field-MS	72.6 µg mL^{-1} for MT-1	[121]
MT-1 and MT-2 isoforms	Rabbit liver MT-1, MT-2 and MT preparations	Capillary zone electrophoresis	Uncoated fused silica capillaries (70-100 cm length, 75 µm id)	5 mM acetate buffer, pH 6 (ICP-MS detection) 10 mM acetate buffer, pH 6 (ESI-MS detection)	UV ICP-MS ESI-triple quadrupole-MS	NR	[146]

Table 5. Analytical methods for the species-selective analysis of proteins tagged with mercury by hyphenated techniques with element-selective detection.

Sample type	Labeling tag	Separation technique	Column	Eluent	Detector	Detection limits	Ref.
Ovalbumin β-lactoglobulin	• CH_3Hg^+ • $CH_3CH_2Hg^+$ • *p*HMB • 2,7-dibromo-4-hydroxymercuri fluorescein	Size exclusion HPLC Reversed phase HPLC	VP-ODS C_{18} (250 mm×2.0 mm id, 5 µm particles) ZORBAX Bio Series GF-250 (250×4.6 mm id, 4-4.5 µm particles)	NR	ICP-dynamic reaction cell-quadrupole-MS ESI-ion trap-MS MALDI-time of flight-MS UV Fluorescence	NR	190
Glutathione Ovalbumin β-lactoglobulin	• CH_3Hg-thiosalicylate • $CH_3{}^{204}Hg$-thiosalicylate	Reversed phase HPLC Size exclusion HPLC	*RPC*: VP-ODS C18 (250 mm×2.0 mm id) *SEC*: Superdex 75 10/300 GL (300 mm×10 mm id, 13 µm particles)	*RPC*: (A): water/0.05% trifluoroacetic acid (B): acetonitrile/0.05% trifluoroacetic acid Gradient: from 30-90% B SEC: 5 mM Tris + 100 mM ammonium bicarbonate, pH 7.35	ICP-MS UV ESI-ion trap-MS ESI-time of flight-MS	45.4 pmol L^{-1} (Glutathione) 15.1 pmol L^{-1} (Ovalbumin) 45.4 pmol L^{-1} (Beta lactoglobulin)	169
Ovalbumin	*p*HMB	Reversed phase µHPLC	Diphenyl (150 mm×300 µm id. 5 µm particles, 300 Å)	(A): 2% acetonitrile/98% water/0.05% trifluoroacetic acid (B): 98% acetonitrile/2% water/0.05% trifluoroacetic acid Gradient: from 20-80%	ICP-MS MALDI-time of flight-MS ESI-time of flight-MS	1 fmol abs. for Ovalbumin	191

Insulin	*p*HMB	Reversed phase μHPLC	Diphenyl (150 mm×300 μm id. 5 μm particles, 300 Å)	B (A): 2% acetonitrile/98% water/0.05% trifluoroacetic acid (B): 98% acetonitrile/2% water/0.05% trifluoroacetic acid Gradient from 0-80% B	ICP-MS MALDI-time of flight-MS ESI-time of flight-MS	subfemtomole	[170]
Bovine pancreatic ribonuclease A Lysozyme Insulin	CH_3Hg^+	Reversed phase HPLC	Zorbax 300SB-C_{18} (150 mm×1 mm id, 3.5 μm particles)	(A): water/0.3% acetic acid (B): acetonitrile/0.3% acetic acid Gradient: from 10-45% B	ICP-dynamic reaction cell-MS ESI-ion trap-MS	0.6 pmol (ribonuclease) 1.2 pmol (lysozyme) 0.4 pmol (insulin)	[192]
Glutathione Phytochelatins, Lysozyme β-lactoglobulin	• CH_3Hg^+ • $CH_3CH_2Hg^+$ • *p*HMB	Reversed phase HPLC	Zorbax C_{18} (150 mm×1 mm id, 3.5 μm particles)	(A): water/0.3% acetic acid (B): acetonitrile/0.3% acetic acid Gradient: from 10-60% B	ESI-ion trap-MS	NR	[19]
Glyceraldehyde-3-phosphate dehydrogenase Aldolase Pyruvate kinase Triose phosphate isomerase Phosphoglucose isomerase	*p*HMB	Hydrophobic interaction HPLC	TSK-Gel1 Ether-5PW (750 mm×7.5 mm id)	(A): 0.05 M phosphate buffer + 1.8 M sodium sulphate, pH 7.2 (B): 0.05 M phosphate buffer, pH 7.2 Gradient: from 0-100% B in 20 min	CVG-AFS	10^{-8} – 10^{-9} mol L^{-1}	[161]
MTs from rabbit liver	*p*HMB	Reversed phase HPLC	RP C_4 (150 mm×4.6 mm id, 5 μm particles, 300 Å)	(A): 95% water/0.1% trifluoroacetic acid/5% methanol	CVG-AFS	0.86 nmol L^{-1} for denaturated	[162]

				(B): 95% methanol/ 0.1% trifluoroacetic acid/5% water Gradient: from 0-50% B in 25 min		MTs	
Phytochelatins Extracts of cell cultures from *Phaeodactylum tricornutum*	*p*HMB	Size exclusion HPLC Reversed phase HPLC	*SEC*: BioSep-SEC-S 2000 (300 mm×7.8 mm, 5 µm particles) *RPC*: Ultrasphere ODS C_{18} (250 mm×4.6 mm, 5 µm particles)	*SEC*: 0.1 M phosphate buffer, pH 6.77 *RPC*: (A): water/0.1% trifluoroacetic acid (B): 5% A + 95% methanol Gradient: from 10-45% B in 30 min, 45-80% B in 25 min	UV CVG-AFS MALDI-time of flight-MS	0.97 ng of glutathione	[193]
Cysteine Glutathione Homocysteine Cysteinyl-glycine	*p*HMB	Reversed phase HPLC	RP C_{12} (150 mm×4.6 mm id, 4 µm particles).	(A): 90% water/ 0.01% trifluoroacetic acid/10% methanol (B): methanol Gradient: from 0-30% B in 15 min	CVG-AFS	45 nmol L^{-1} for all the species	[182]
S-nitrosoglutathione in human blood	*p*HMB	Reversed phase HPLC	Hydra RP C_{18} (250 mm×4.6 mm, 4 µm particles)	99% 0.02 M phosphate buffer pH 6.0, 1% methanol	CVG-AFS	25 nmol L^{-1}	[166]
Human serum albumin Bovine serum albumin Rat serum albumin Horse serum albumin Sheep serum	*p*HMB	Reversed phase HPLC	RP C_4 (150 mm×4.6 mm id, 5 µm particles, 300 Å)	(A): water/0.1% trifluoroacetic acid/40% methanol (B): methanol/0.1% trifluoroacetic acid/10% water Gradient: from 0-100% B in 43 min	CVG-AFS	57 nmol L^{-1} for *p*HMB	[194]

albumin
Ovalbumin
β-lactoglobulin

Table 6. Analytical methods for the species-selective analysis of proteins tagged with iodine and ferrocene by hyphenated techniques with element-selective detection.

Sample type	Labeling tag	Separation technique	Column	Eluent	Detector	Detection limits	Ref.
Tyrosine Three peptides from reference material (NIST 8327) Tryptic digests of β-casein	Bis(pyridine)iodonium tetrafluoroborate	Reversed phase capillary HPLC	Zorbax SB C_{18} (150 mm×0.3 mm, 5 μm particles, 80 Å)	(A): water/0.1% formic acid/50 ng Ge mL^{-1} (B): acetonitrile/0.1% formic acid/50 ng Ge mL^{-1} Gradient for tyrosine: 3% B for 6.5 min, 3-80% B in 23 min, 80% B for 2 min Gradient for peptides: 5% B for 6.5 min, 3-80% B in 23 min, 80% B for 2 min Gradient for tryptic digests of β-casein: 5% B for 6.5 min, 5-20% B in 7 min, 20-40% B in 49.5 min, 40-80% B in 5 min, 80-95% B in 2 min, 95% B for 1 min	ICP-collision cell-MS ESI-quadrupole/time of flight-MS	480 pmol L^{-1}, 480 amol absolute	[196]
Cytochromes P450	Monoclonal antibody labeled with iodine	SDS-PAGE Semidry immunoblot	-	Rehydration buffer: 0.3 M Tris/acetate, 0.1% SDS, 0.01% sodium azide, pH 8.0 Anode buffer: 0.3 M Tris/acetate, 0.1% SDS, 0.01% sodium azide, pH 8.4 Cathode buffer: 0.8 M Ntris(hydroxymethyl)methylglycine, 0.08 M Tris, 0.1% SDS, 0.01% sodium azide, pH 7.15) Sample buffer 1 mL 0.5 M Tris	LA-ICP-MS	70 fmol for CYP2E1	[218]

Sample type	Labeling tag	Separation technique	Column	Eluent	Detector	Detection limits	Ref.
Lysozyme Bovine serum albumin Cytochrome c β-casein	Potassium triiodide IODO-Beads	SDS-PAGE Semidry blotting Western blotting Reversed phase HPLC	ZIC-HILIC microbore (150 mm×1 mm id, 3.5 μm particles) + guard (5 mm×1 mm id, 5 μm particles)	pH 7.5, 0.1 g SDS, 15.5 mg DTT, made upto 10 mL with water *RPC*: (A): 5% water/95% acetonitrile + 10 mM ammonium acetate/ acetic acid, pH 4.8 (B): 5% acetonitrile/95% water + 10 mM ammonium acetate/ acetic acid, pH 4.8 Gradient: 10% B for 2 min, 10-80% B in 36 min, 80% B for 7 min	LA-ICP-sector field-MS ESI-linear triple quadrupole/ Fourier transform- MS	low pmol/upper fmol-range	[205]
Porcine gastric mucosa pepsin Lysozyme Bovine serum albumin	Sodium iodide	SDS-PAGE Semidry blotting	-	Sample buffer solution: 8 mL of water + 2 mL of 0.5 M Tris (pH 6.8), 1.6 mL of 80% glycerol + 3.2 mL of 10% (w/v) SDS and 23.4 mg of 1,4-dithio-DL-threitol.	LA-ICP-sector field-MS nanoESI-Fourier transform ion cyclotron resonance-MS	150 fmol for bovine serum albumin	[198]
Lysozyme β-lactoglobulin A Insulin	• Succinimidylferrocenyl propionate (for amino groups) • Ferrocenecarboxylic acid(2-maleimidoyl)ethyl amide (for thiolic groups)	Reversed phase HPLC	BioWidePore C_5 (150 mm×2.1 mm id, 5 μm particles)	(A): water/0.1% formic acid (B): acetonitrile Gradient A: 10% B for 1 min, 10-100%B in 4 min, 100% B for 1 min Gradient B: 10% B for 1 min, 10-100%B in 14 min, 100% B for 2 min	ICP-octapole reaction cell-MS ESI-quadrupole/ion trap-MS	NR	[212]
Haemoglobin A_{1c}	Ferrocene-conjugated anti-human haemoglobin monoclonal antibody	On-chip type cation-exchange chromatograph	On-chip type column (depth: 1mm; width: 1mm; length: 30mm) in	(A): 20mM sodium acetate, pH 5.5 (B): 200mM NaCl, pH 5.5 Gradient from 0-100% B in 30	Electrochemical detector	NR	[217]

Sample type	Labeling tag	Separation technique	Column	Eluent	Detector	Detection limits	Ref.
		y Cation exchange HPLC	polymethylmethacrylate, packed with cation-exchange beads (particle diameter: 30µm) *CEC:* Tricon column (20 x 5mm) filled with 700µL of cation-exchange resin beads (SOURCE 30S; spherical and porous bead (30µm diameter); matrix polystyrene/divinyl benzene; type of ion exchanger: $R–O–CH_2–CHOH–CH_2–O–CH_2–CHOH–CH_2–SO_3^-$)	min			
Lysozyme β-lactoglobulin A Insulin	N-(2-Ferroceneethyl)maleimide	Reversed phase HPLC	Discovery BioWidePore C5-5 (150 mm×2.1 mm id, 5 µm particles)	(A): 0.1% formic acid in water/0.1% formic acid in acetonitrile (B): 10 mM NH_4HCO_3 buffer and acetonitrile Gradient A: from 10-100% acetonitrile in 20 min Gradient B: from 10-100% acetonitrile in 25 min Gradient C: from 5-60% acetonitrile in 5 min, 60-75% acetonitrile in 15 min, 75-100%	Cyclic voltammetry hyphenated with a single-quadrupole-MS	NR	[219]

Sample type	Labeling tag	Separation technique	Column	Eluent	Detector	Detection limits	Ref.
				acetonitrile in 2 min (A): 10 mM ammonium formate, pH 4 (B): acetonitrile Gradient A: 5-33% B in 5 min, 33-40% B in 20 min, 40-90% B in 3 min Gradient B: 30-40% B in 5 min, 40-50%B in 20 min, 50-90% B in 3 min Gradient C: 5-90% B in 20 min			
α-lactalbumin β-lactoglobulin B β-lactoglobulin A	N-(2-ferrocene-ethyl)maleimide Ferrocenecarboxylic acid-(2-maleimidoyl)ethylamide	Reversed phase HPLC	Discovery BioWidePore C5-5 (150 mm×2.1 mm id, 5 µm particles)		Cyclic voltammetry hyphenated with ESI-quadrupole/ion trap-MS	NR	[207]
Phytochelatins from algae extracts	Ferrocenecarboxylic acid (2-maleimidoyl)ethylamide	Reversed phase HPLC	Discovery C8 (150 mm×2.1 mm id, 5 µm particles, 180 Å)	(A): 0.1% formic acid/water (B): acetonitrile Gradient: 5% B, 5-90% B in 60 min, 90% B for 3 min	ESI-time of flight-MS ESI-Triple quadrupole/ion trap-MS ICP-MS	0.69 µmol L^{-1} for $CysPC_2$ 0.59 µmol L^{-1} for PC_2	[216]

Table 7. Analytical methods for the species-selective analysis of proteins tagged with MeCAT by hyphenated techniques with element-selective detection.

Sample type	Labeling tag	Separation technique	Column	Eluent	Detector	Detection limits	Ref.
Vasopressin GGYGGC Somatostatin	• MMA-DOTA loaded with Eu(III) Fluorescein isothiocycanate	Reversed phase HPLC Capillary electrophoresis	Zorbax 300SB-C_{18} column (150 mm × 1 mm id, 3.5μm particles) Uncoated fused-silica capillary (40 cm length, 75 μm id)	*RPC*: (A): water/0.05% trifluoroacetic acid (B): acetonitrile/0.05% trifluoroacetic acid Gradient: from 25-60% B in 30 min *CE*: Running buffer 200 mM sodium carbonate-sodium biacarbonate, pH 9.5	UV ESI-ion trap-MS ICP-MS CE-LIF	0.130 nmol L^{-1} Vasopressin 0.130 nmol L^{-1} Somatostatin 0.259 nmol L^{-1} GGYGGC	235
Sus scrofa eye lens proteins α-lactalbumin Bovine serum albumin	DOTA loaded with Lu(III), Ho(III), Tb(III), Tm(III)	1-D SDS-PAGE 2-D SDS-PAGE Reversed phase nanoHPCL	Zorbax 300SB-C_{18} (150 mm×75 μm id, 3.5 μm particles) + guard Zorbax 300SB-C_{18} (0.3 mm×5 mm, 3.5 μm particles)	*RPC*: (A): 98.5% water/1% acetonitrile/0.5% formic acid (B): 94.9% water/5% acetonitrile/0.1% formic acid (C): 99.9% acetonitrile/0.1% formic acid	ICP-quadrupole-MS ICP-high resolution sector field-MS MALDI-time of flight-MS ESI-time of flight-MS	110 amol bovine serum albumin 670 amol alpha lactalbumin	225
Insulin Insulin chain A Insulin chain B	DTPA loaded with Lu(III)	Reversed phase nanoHPCL	Acclaim PepMap100 C_{18} (15 cm×75 μm id, 3 μm particles, 100 Å)	(A): 10 mM triethylammonium bicarbonate, pH 7.5 (B): 90% acetonitrile + 10% 100 mM triethylammonium	ICP-MS ESI-quadrupole/time of flight-MS	179 amol for tagged proteins	229

				bicarbonate, pH 7.5 Gradient for standard peptides: 5-100% B in 15 min Gradient for tryptic digests: 5% B for 5 min, 5-62% B in 10 min, 62-100% B in 2 min, 100% B for 5 min			
Lysozyme Bovine serum albumin	MeCAT-Eu (Proteome Factory AG, Berlin, Germany)	Reversed phase HPCL	RP-C_{18} column (Polaris 3 C18-Ether, 150 mm×1 mm id, 5 µm particles).	(A): water/1% ACN/0.1% formic acid (B): 90% ACN/0.1% formic acid/10% water Gradient A: from 5-15% B in 1 min, 15-20% B in 2 min, 20-23% B in 35 min, 23-50% B in 3 min Gradient B: from 15-18% B in 10 min, 18-25% B in 60 min, 25-40% B in 2 min	ICP-MS	1.6 fmol for tagged proteins	[7]
Lysozyme Insulin Ribonuclease A	MMA-DOTA loaded with Eu(III)	Reversed phase HPCL	Zorbax 300SB C_{18} (150 mm×1 mm id; 3.5 µm particles).	(A): water/0.05% trifluoroacetic acid (B): acetonitrile/0.05% trifluoroacetic acid Gradient A: from 20-70% B in 13 min Gradient B: from 20-60% B in 30 min Gradient C: from 5-60% C in 55 min)	ICP-MS ESI-ion trap-MS	0.819 fmol for lysozyme	[224]
Bradykinin Substance P	DTPA loaded with Eu(III)	Reversed phase HPCL	Luna C18(2)	(A): water/0.05% formic acid (B): acetonitrile/0.05%	ICP-quadrupole-MS UV	1.0×10^7 fmol	[241]

				formic acid Gradient A: from 0-33% B in 20 min Gradient B: from 0-15% B in 5 min, 15-18.75% B in 15 min	ESI-MS/MS		
$B\beta_{15-42}$	DOTA loaded with In(III)	Reversed phase HPCL	Nucleosil C_8 (150mm×1 mm, 5 µm particles; 100 Å)	(A): water/0.1 % formic acid/1% methanol (B): methanol/0.1 % formic acid/1 water Gradient: 10% B for 4 min, 10-100%B in 1 min, 100% B for 5 min	ICP-dynamic reaction cell-MS ESI-time of flight-MS	1.7 fmol	[240]
Bovine serum albumin Lysozyme	DOTA loaded with lanthanides	SDS-PAGE Semidry blotting	-	-	LA-ICP-MS nanoESI-ion cyclotron resonance Fourier transform -MS	15 fmol	[204]
Bradykinin	DOTA-NHS-ester loaded with Eu(III)	Reversed phase HPLC Gas chromatography	ZORBAX Eclipse Plus C_{18} (150 mm×2.1 mm, 5 µm particles) DB-5MS (30 m×0.25 µm)	*RPC*: (A): water (B): acetonitrile Gradient: 95% A for 5 minutes; from 5-40% B 35 min *GC*: Carrier gas helium	ICP-quadrupole-MS ESI-ion trap-MS MS GC-MS	7.2 fmol	[242]
Aprotinin::β-galactosidase fusion protein	MeCAT reagent loaded with Ho(III) and Lu(III)	2-D SDS-PAGE Reversed phase nanoHPLC	Zorbax 300 SB-C_{18} (150 mm×75 µm id, 3.5 µm particles) + guard Zorbax 300 SB-C_{18} (0.3 mm×5 mm, 3.5 µm particles)	(A): 94.9% water/5% acetonitrile/0.1% formic acid (B): 99.9% acetonitrile/0.1% formic acid Gradient A: from 0-100% B in 45 min	ICP-MS ESI-quadrupole/time of flight-MS	NR	[243]

Bovine serum albumin *Escherichia coli* cell lysate	MeCAT reagent loaded with Tb(III), Ho(III), Tm(III), Lu(III)	Reversed phase nanoHPLC	Zorbax 300 SB-C_{18} (150 mm×75 µm id, 3.5 µm particles) + guard Zorbax 300 SB-C_{18} (0.3 mm×5 mm, 3.5 µm particles)	Gradient B: from 0-100% B in 75 min (A): 94.9% water/5% acetonitrile/0.1% formic acid (B): 99.9% acetonitrile/0.1% formic acid Gradient A: from 0-100% B in 45 min	ESI- linear triple quadrupole/ion cyclotron resonance Fourier transform -MS	Lower than 10 fmol	228
Bovine serum albumin Human serum albumin Cysteine-containing synthetic standard peptides	MeCAT reagent and DOTA-NHS ester loaded with lanthanides	Reversed phase nanoHPLC	Zorbax 300 SB-C_{18} (150 mm×75 µm id, 3.5 µm particles) + guard Zorbax 300 SB-C_{18} (0.3 mm×5 mm, 3.5 µm particles) Zorbax SB-C_{18} (150 mm×0.5 mm id, 5 µm particles)	Gradient B: from 0-100% B in 75 min (A): water/5% acetonitrile/0.1% formic acid (B): 99.9% acetonitrile/0.1% formic acid Gradient: from 0-100% B in 35 min (A): water/1% acetonitrile/0.1% formic acid (B): 90% acetonitrile/0.1% formic acid/10% water Gradient: from 0-100% B in 75 min	ESI-ion cyclotron resonance Fourier transform -MS ICP-MS	1.6 fmol for MeCAT labeled peptides	236
Bovine serum albumin Ovalbumin β -casein Proteinaceous	DTPA loaded with Eu(III)	Reversed phase HPLC	Symmetry300 C_{18} (100 mm×2.1 mm id)	(A): water/0.05% trifluoroacetic acid (B): acetonitrile/0.05% TFA Gradient: 5% B for 3	ICP-MS MALDI-time of flight-MS	NR	244

binders (animal glue, egg yolk, egg white, whole egg, casein) Vasopressin Oxytocin RNase A Somatostatin Cytochrome C Lysozyme Bovine serum albumin Chymotrypsin Elastase Carbonic anhydrase				min, 5-45% B in 7 min, 45% B for 6 min, 45-70% B in 9 min			
Lysozyme Bovine serum albumin Chymotrypsin Elastase Carbonic anhydrase	azido-DOTA loaded with Eu(III)	Reversed phase HPLC	Zorbax 300SB-C_{18} column (150 mm × 1 mm id, 3.5μm particles)	(A): water/0.05% trifluoroacetic acid (B): acetonitrile/0.05% trifluoroacetic acid Gradient: from 5-60% B in 45 min	ESI-ion trap-MS ICP-MS	0.2 fmol	[245]
α-lactalbumin Lysozyme	MeCAT-iodoacetamide reagent loaded with Eu(III), Tb(III), Lu(III), Tm(III)	SDS-PAGE Reversed phase nanoHPLC Reversed phase HPLC	*RPC*: Polaris 3 C_{18}-Ether (150 mm×1 mm id) *nanoRPC:* Zorbax 300 SB-C_{18} (150 mm×75 μm id, 3.5 μm particle) + guard Zorbax 300 SB-C_{18} (0.3 mm×5 mm, 3.5 μm particle)	*RPC* and *nanoRPC*: (A): 94.4% water/5% acetonitrile/0.1% formic acid (B): 99.9% acetonitrile/0.1% formic acid Gradient: from 0-100% B in 35 min	ICP-MS ESI-linear triple quadrupole/ion cyclotron resonance Fourier transform-MS	NR	[223]
Lysozyme Human serum albumin Transferrin Human serum samples	MeCAT-iodoacetamide reagent loaded with Yb(III)	SDS-PAGE	-	NR	LA-ICP-MS	750 amol for spot mineralization 15 fmol for direct LA	[246]
Lysozyme Bovine serum	MeCAT reagent loaded with	2-D strong cation exchange	*CEC*: BioBasic SCX (150	*CEC*: 0 – 4 – 10 – 120 – 200	ICP-MS ESI- linear triple	Below 300 amol for any MeCAT-	[247]

albumin Transferrin	Eu(III), Ho(III), Lu(III), Tm(III)	and reversed-phase HPLC	mm×1 mm id, 5 μm particles) *RPC*: Polaris 3 C_{18}-Ether (150mm×1 mm id, 5 μm particles)	mM ammomium acetate *RPC*: (A): water/1% acetonitrile/0.1% formic acid (B): 90% acetonitrile/0.1% formic acid/10% water Gradient: 100% A for 5 min, 0-10% B in 31 min, 10-20% B in 39 min, 20-40% B in 10 min, 40-80% B in 1 min	quadrupole/ion cyclotron resonance Fourier transform -MS	labeled peptide	
β-lactoglobulin Bovine serum albumin	MeCAT-iodoacetamide reagent loaded with Ho(III)	Reversed phase nanoHPLC	Zorbax 300 SB-C_{18} (150 mm×75 μm id, 3.5 μm particles) + guard Zorbax 300 SB-C_{18} (0.3 mm×5 mm, 3.5 μm particles)	(A): 94.4% water/5% acetonitrile/0.1% formic acid (B): 99.9% acetonitrile/0.1% formic acid Gradient: from 0-100% B in 50 min	ESI- linear triple quadrupole/ion cyclotron resonance Fourier transform -MS	NR	[248]
Synthetic model peptides	DOTA-NHS ester loaded with Tm(III) and Tb(III)	Reversed phase HPLC	GEAgel SP-300-ODS-AP (150 mm×75 μm id, 5 μm particles)	(A): 98% water/2% acetonitrile/0.1% fomic acid (B): 20% water/80% acetonitrile/0.1% formic acid Gradient: 100% A for 15 min, 2-50% B in 15 min	MALDI-time of flight-MS ESI- linear triple quadrupole/ion cyclotron resonance Fourier transform -MS Nanospray source-quadrupole/ion trap-MS	0.8 fmol L^{-1}	[249]
Bovine serum albumin α-lactalbumin	DTPA loaded with Y(III) and Tb(III)	Reversed phase HPLC	PepMap C18 column (150 mm×75 μm id)	(A): 95% water/0.1% formic acid/5%acetonitrile	MALDI-time of flight-MS ESI-	NR	[221]

β-lactoglobin Myoglobin (nonapo form) Lysozyme Bovine apotransferrin Bovine insulin				(B): 85% acetonitrile/0.1% formic acid/15% water Gradient: 4% B for 4.5 min, 4-50% B in 45 min; 50-100% B in 10 min; 100% B for 10 min.	quadrupole/time of flight-MS		
RNase A Cytochrome c Lysozyme	DTPAA loaded with Ce(III) and Sm(III)	Cation exchange HPLC	TSKGEL SP-5PW (75 mm×7.5 mm id, 10 μm particle)	(A): 0.02 M phosphate buffer, pH 6.5 (B): A + 0.5 M ammonium chloride Gradient: from 24-55% B in 16 min, 55-100% B in 14 min	ICP-MS	0.2 pmol (RNase A) 1 pmol (cytochrome c) 7 pmol (lysozyme)	230
Synthetic model peptides Lysozyme	DOTA-NHS-ester loaded with Ho(III), Tm(III), Lu(III), Er(III)	Nano ion pairing reversed-phase HPLC	Acclaim PepMap100 C_{18} (150 mm×75 μm id, 5 μm particles)	(A): water/0.05% trifluoroacetic acid (B): 80% acetonitrile/0.04% trifluoroacetic acid/20% water Gradient: 100% A for 6 min, 5-70% B in 20 min; 70-95% B in 1 min; 95% B for 10 min Gradient for digests: 100% A for 6 min, 5-70% B in 40 min; 70-95% B in 1 min; 95% B for 10 min	ICP-MS MALDI-time of flight/time of flight-MS	NR	222
Myoglobin Transferrin Thyroglobulin	Ru-NHS ester	Size exclusion HPLC	Superdex 200 10/300 GL (300 mm×10 mm id)	200 mM ammonium acetate buffer, pH 7.2	ICP-quadrupole-MS ICP-sector field-MS	1.6 fmol (myoglobin) 3.2 fmol (transferrin) 7.0 fmol	250

					(thyroglobulin)	
Angiotensin I Angiotensin II Bradykinin MARCKS peptide clip	DOTA-NHS-ester loaded with Tb(III), Tm(III) and Ho(III)	Reversed phase HPLC	Luna C18(2) HST (2.5 mm×3 mm ×100 mm)	98.5% water/1.5% acetonitrile (B): 1.5% water/98.5% acetonitrile Gradient: from 0-100% B in 24 min	ICP-collision cell-MS MALDI-MS ESI-quadrupole/ion trap-MS	NR	[251]
r-fetoprotein Human chorionic gonadotropin Carcinoembryonic antigen Ovarian tumor antigen Gastrointestinal tumor antigen	Antibodies labeled with Pr(III), Eu(III), Gd(III), Ho(III), and Tb(III)	Size exclusion HPLC	Superdex 200 HR 10/30 (300 mm× 10 mm id, 13 μm particles)	100 mM ammonium acetate, pH 6.8	UV ICP-MS	Ranging from 2.6 to 8.5 ng mL^{-1}	[237]