MEDNARODNA PODIPLOMSKA ŠOLA JOŽEFA STEFANA

JOŽEF STEFAN INTERNATIONAL POSTGRADUATE SCHOOL

A novel method for speciation of Pt in human serum by conjoint liquid chromatography on monolithic disks with UV and ICP-MS detection

Anže Martinčič*#, Radmila Milačič*#, Maja Čemažar**, Gregor Serša** and Janez Ščančar*#

*Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia
#Josef Stefan International Postgraduate School, Jamova 39, 1000, Ljubljana
**Institute of Oncology, Zaloška cesta 2, 1000 Ljubljana, Slovenia

INTRODUCTION

In speciation analysis of platinum in human serum, ion exchange (IE) chromatography is frequently used. As an alternative to classic – particle packed IE columns, monolithic supports have been successfully applied. They have several advantages over classical columns (e.g. greater robustness) but both are unable to separate unbound drug from immunoglobulins (IgG), which co-elute at the same retention time t_0 (Figure 1) [1]. To overcome this problem a conjoint liquid chromatography (CLC) column containing one CIM Protein G and one CIM DEAE monolithic disk in a single housing was constructed. Such set up allows two dimensional separation in one chromatographic run. CIM Protein G disk (affinity chromatography) binds Fc region of IgG thus separating free Pt-based drug from portion that is bound to IgG, while on a weak anion exchange CIM DEAE disk serum transferrin and albumin were separated.

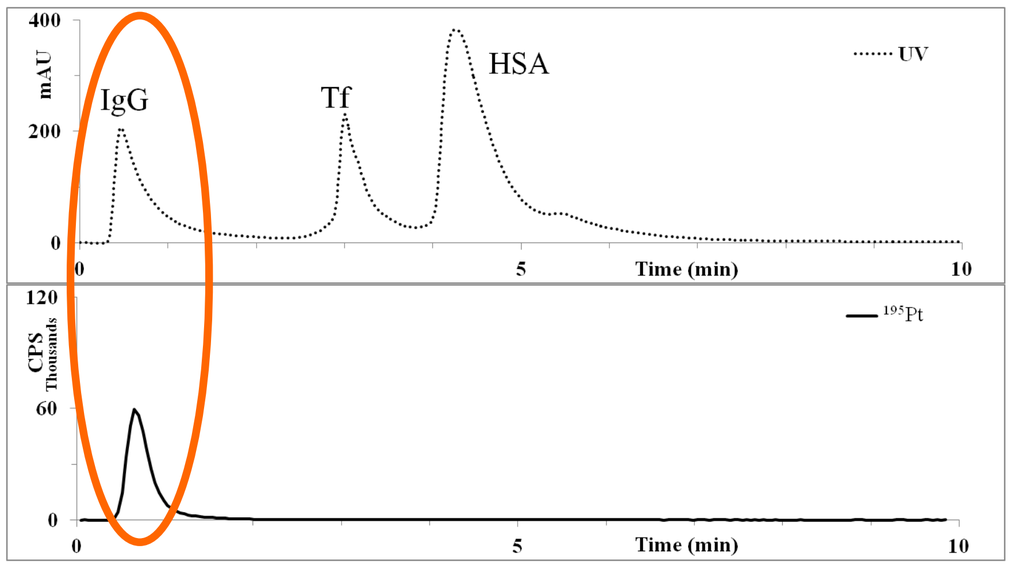

Figure 1: Co-elution of unbound drug and IgG. Chromatogram of the mixture of serum proteins (above). Chromatogram of cisplatin diluted in buffer A (below).

METHODS

Chromatographic separation on Agilent 1200 HPLC and elemental detection by Agilent 7700x ICP-MS

1.CIM DEAE and CIM protein G disks

- 0.05 M Tris HCl + 0.03 M $NaHCO_3$, pH 7.4 (buffer A)
- 0.05 M Tris HCl + 1 M NH_4Cl, pH 7.4 (buffer B)
- 0.5 M AcOH, (eluent C)
- Sample volume: 0.1 mL
- Flow 1 mL/min
- Analysis time: 14 min; 1st min isocratic at 100 % buffer A, next 9 min gradient elution from 100 % A to 100 % B, and the last 4 min eluent C.
- Column regeneration time: 9 min

Figure 2: CIM Protein G disk column

2.UV detection at 278 nm

3.ICP-MS

- RF power: 1550 W
- Carrier gas: 0.35 L/min, dilution gas: 0.82 L/min
- Integration time on m/z 195 and 194: 0.7 s
- Time resolved analysis: 14 min

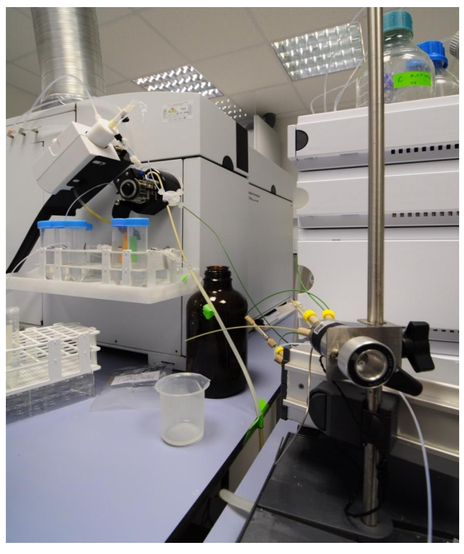

Figure 3: Instrument setup

RESULTS AND DISSCUSSION

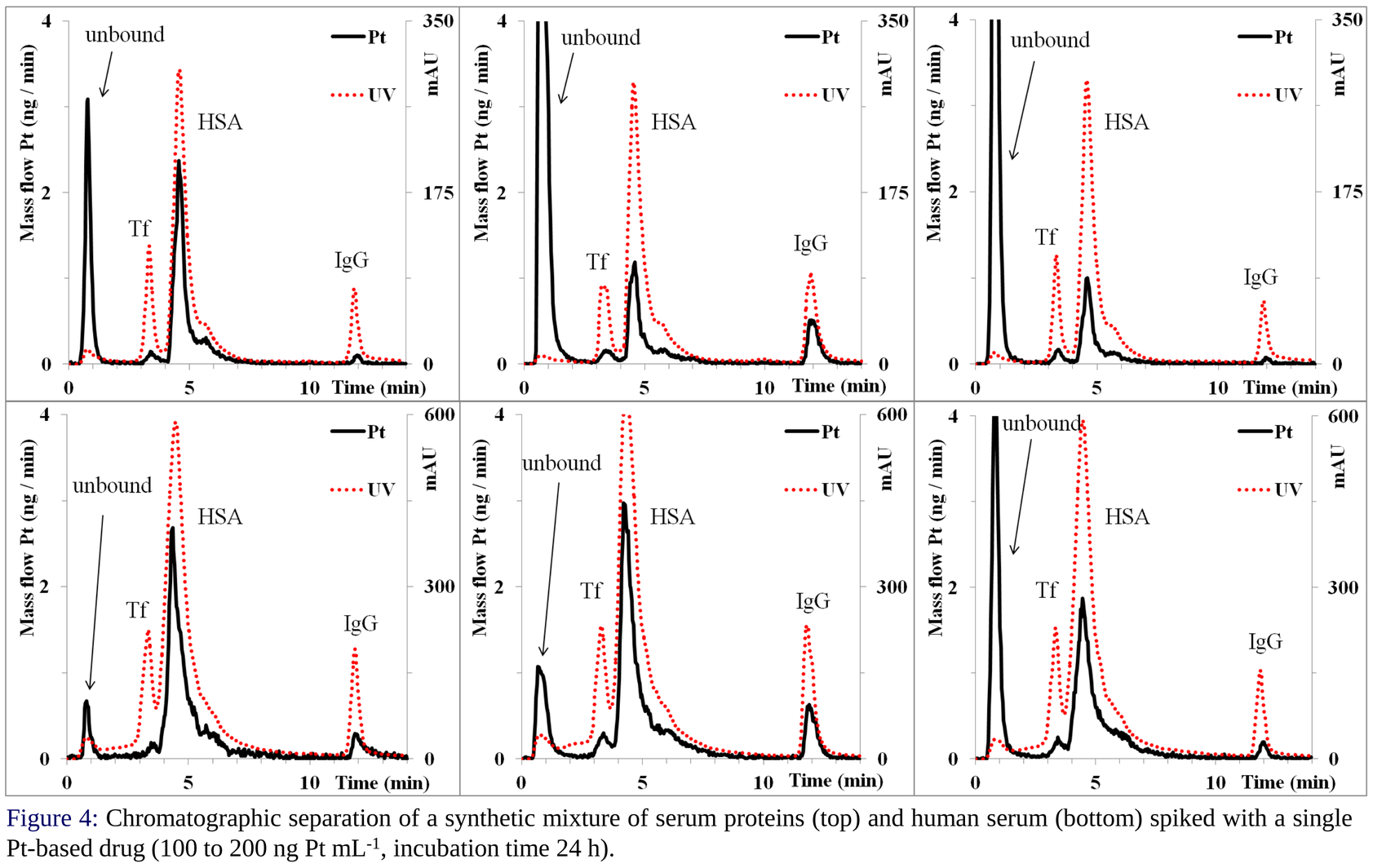

Figure 4: Chromatographic separation of a synthetic mixture of serum proteins (top) and human serum (bottom) spiked with a single Pt-based drug (100 to 200 ng Pt mL^{-1}, incubation time 24 h).

As can be seen from Figure 2, the developed method separates all the major Pt species found in human serum. The use of post-column ID adequately compensated the changes in eluent composition, while also providing the means for quantifying our results (table 1).

Table 1: Distribution of Pt in human serum

Pt drug	Incubation time (h)	Pt spike added	Unbound Pt	Pt bound to Tf	Pt bound to HSA	Pt bound to IgG	Column recovery
		(ng mL^{-1})	(ng mL^{-1})	(ng mL^{-1})	(ng mL^{-1})	(ng mL^{-1})	(%)
Cisplatin	24 h	137.4±1.1	19.5±0.1	2.50±0.02	103.5±0.8	5.00±0.04	95
Oxaliplatin	24 h	153.7±1.2	27.0±0.2	4.01±0.03	102.1±0.8	16.0±0.1	97
Carboplatin	48 h	165.6±1.3	77.5±0.6	3.02±0.02	75.5±0.6	3.01±0.03	96

CONCLUSIONS

The developed method is uniquely able to separate unbound drug from drug bound to IgG while preserving all the advantages of monolithic chromatography, mainly speed and robustness.