Quantification of Cisplatin via ZIC®-HILIC Separation and on-line ICP-MS Detection

Cisplatin can be readily determined using ZIC®-HILIC separation and ICP-MS detection using DMF as organic modifier in the mobile phase. The combination of a low-volatile modifier and a sample introduction system for partial aerosol desolvation provided excellent sensitivity and minimized system maintenance.

Introduction

HPLC separation with ICP-MS detection is an very powerful tool in the field of metallomics, the characterization of metals and metallic compounds in cells and tissues. The technique can provide detailed information about cell processes and has been used with a broad range of techniques including reversed phase (RP), ion exchange (IEC) and chiral chromatography. Recently, it has been demonstrated that hydrophilic interaction chromatography can be coupled to ICP-MS using dimethylformamide as the organic solvent, thereby extending the use of LC-ICP-MS to the study of very hydrophilic and uncharged species.

Hydrophilic interaction liquid chromatography (HILIC) is a very powerful technique for the separation of complex mixtures of polar compounds. HILIC separates compounds using a mostly organic hydrophobic mobile phase with a hydrophilic stationary phase. The solutes elute in order of increasing hydrophilicity, which is the opposite of the elution order in RP and is especially useful for the separation of polar compounds that are poorly retained by RP.

ZIC®-HILIC chromatography is a unique form of HILIC where a bonded zwitterionic sulfobetaine group on a silica or polymer backbone acts as the interactive layer. The low reactivity and zwitterionic properties of the sulfobetaine group makes the ZIC®-HILIC-ICP-MS a logical choice for the highly reactive and zwitterionic cisplatin.

This note, describes the separation and detection of cisplatin, an antineoplastic medication that interferes with the growth of cancer cells and slows their growth in the body.

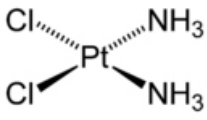

Figure 1: Structure of Cisplatin, an antineoplastic medication that interferes with the growth of cancer cells and slows their growth in the body

Experimental

Reagents and Eluants: Cisplatin (cis-DDP) and eluents (DMF or acetonitrile) were used as received. Eluents were prepared from HPLC grade NH_4OAc and Milli-Q water. The monohydrolyzed metabolite of cis-DDP was obtained by heating an aqueous solution of 0.100 µg/mL cis-DDP at 37 °C for ~ 2 h.

Preparation of Whole Cell Samples: Cells from the T289 human malignant melanoma cell line were grown to 80-90% confluence and then exposed to growth media containing 50 µg/mL cis-DDP. After exposure, cells were washed, incubated in cis-DDP free medium for different times, harvested and lysed in Burans mixture. Before analysis, the cell lysates were centrifuged and the supernatant was treated by cut-off centrifugation to isolate the low-molecular fraction and diluted.

Instrumentation: The samples were separated using LKB 2150 pump which was interfaced to a Perkin-Elmer/Sciex Elam 6000 ICP mass spectrometer for identification and quantification of platinum compounds. The mobile phase was delivered by an LKB 2150 pump at a flow rate of 100 µL/min. An aqueous make up flow was mixed in post column for organic solvent dilution and introduced via PEEK tubing to a MiraMist enhanced parallel path nebulizer. The nebulizer was coupled to an ice-water chilled cyclone spray chamber in series with a heated zone (65°C) and a Peltier condenser (5°C) fitted into an EPOND Super Electron introduction system.

Separation: Samples were separated using a ZIC®-HILIC column (150 x 2.1 mm, 5 µm, 200 Å particles) and a isocratic mobile phase consisting of the appropriate organic modifier and 20 mM aqueous ammonium formate (pH ~6.5) [70:30]

Results

A typical chromatogram showing the separation of cis-DDP and its active hydrolyzed metabolite, MH-DDP, is shown in Figure 2.

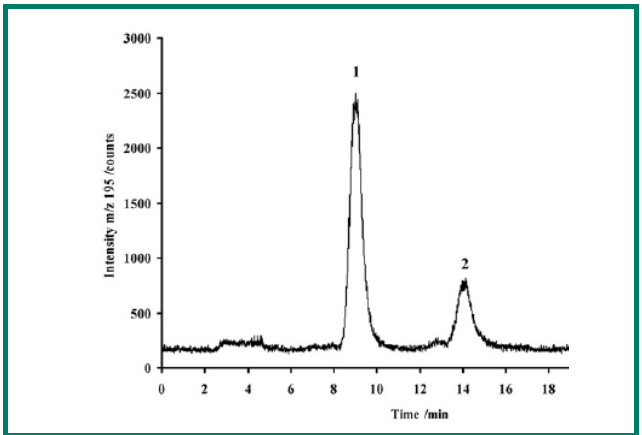

Figure 2: HILIC-ICPMS chromatogram of cis-DDP standards hydrolyzed at 37 °C for 2 h to obtain a cis-DDP (1) and MH-DDP (2) mixture: 10 ng/mL standard eluted with a mobile phase consisting of DMF and 20 mM ammonium acetate (70:30 v/v).

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A similar separation was obtained with acetonitrile (ACN) as the organic component of the mobile phase. The retention times for the two compounds were somewhat longer and the peaks were somewhat sharper as a higher backpressure was employed with DMF, due to its higher viscosity. In addition, when ACN was employed, several non-identified peaks were obtained, which are likely due to the reaction of the mobile phase with cis-DDP and/or MH-DDP.

Analysis of Melanoma Cells Exposed to DDP

The analysis of in-vitro grown cells is useful for elucidating fundamental intracellular processes. However these analyses may be challenging because of the complex matrix composition, a small sample volume and a low concentration of the drug (and its metabolites) in the cell. In Figure 3 we present the chromatogram of a whole cell lysate from in-vitro grown T289 cells that were exposed to 50 µg/mL DDP for 1 hr.

After exposure to cis-DDP, the cis-DDP found in cell lysates was measured in two different cell series, and was found to be 23% and 3.2 % of the total platinum in the two series. The concentration of cis-DDP as a function of incubation time (exposure of the cells loaded with cis-DDP to cis-DDP free medium) was studied. The assay was able to monitor the concentration for a period of 60 min with results presented in table 1.

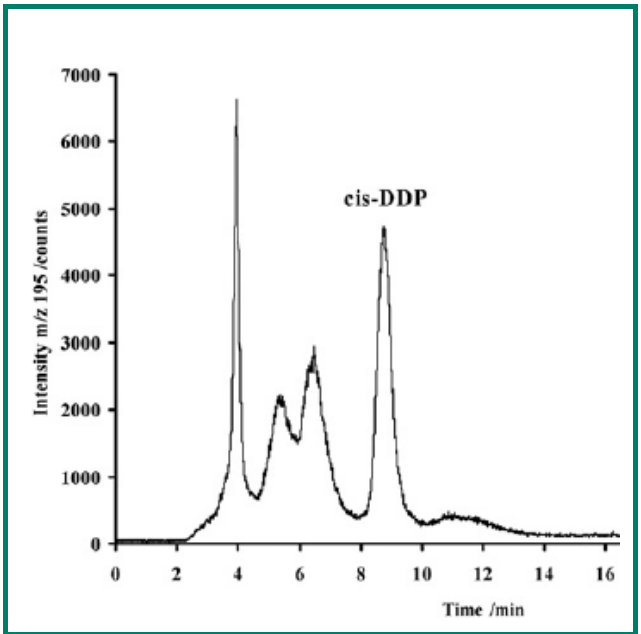

Figure 3: HILIC-ICPMS chromatogram of whole cell lysate from in-vitro grown T289 malignant melanoma cells exposed to 50 µg/ mL cis-DDP for 1 h. Cell lysates were treated by 5 kDa cut-off centrifugation prior to analysis.

When cells were exposed to cis-DDP for only 30 sec, 8.7 ng/mL of Pt as cis-DDP was observed, which corresponded to ca 10 % of the amount after 1 h exposure (this may be due to intracellular cis-DDP or simply bonded to the cell wall, while the major amount of cis-DDP after one hour exposure is intracellular). These results suggest that further studies of cellular uptake and intracellular reaction kinetics of cis-DDP via HILIC-ICPMS is warranted.

Incubation time/min	Total Pt/ng Pt/mL	DDP/ng Pt/mL	%-DDP of total Pt
0	500	110	23
15	620	64	10
30	580	16	2.7
60	650	6.8	1.0
90	510	<3	
120	600	<3	
240	540	<3	
Control[a],[c]	22	8.7	39
0[b]	525	15	2.9
0[b]	445	18	4.0
0[b]	530	15	2.8
(%RSD)	9.5	11	21

a), b) Refer to different samples prepared on different days.
c) Control sample was exposed to 50 µg/mL cis-DDP for 30 sec.

Table 1: Total Platinum and cis-DDP concentrations in whole cell lysates of in-vitro grown T289 human malignant melanoma cells exposed to 50 µg/mL cis-DDP in growth medium for 1 h followed by incubation in cis-DDP free medium for different times

Conclusions

HILIC with ICPMS is a superb technique for measuring the concentration of very hydrophilic species in cells and is a powerful tool for metallomics. A simple, robust and rapid separation was developed for the determination of cis-DDP and MH-DDP in cells with excellent sensitivity which can provide insight into various intracellular processes

About ZIC®-HILIC Chromatography

ZIC®-HILIC stationary phases are based on the covalently bonded permanent zwitterionic sulfobetaine group shown in Figure 4. It is available with a silica support in 3.5, 5 and 10 µm particle sizes in various column dimensions from capillary to semi-preparative (75 µm up to 20 mm ID). In addition, it is available with a polymeric support in 5 µm particles (ZIC®-*p*HILIC). Merck SeQuant also publishes the tutorial booklet A Practical Guide to HILIC, which is available free of charge.

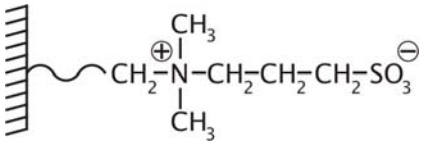

Figure 4: The Bonded Zwitterionic Sulfobetaine Group of ZIC®-HILIC.

About Merck SeQuant

Merck SeQuant AB is a Swedish company that develops a broad range of innovative products for separation and purification of complex samples via chromatography. The company was founded in 1987 by researchers from Umeå University.

Reference

Note: This application note is condensed from the scientific paper "*Hydrophillic interaction liquid chromatography (HILIC) coupled to inductively coupled plasma mass spectrometry (ICPMS) using a mobile phase with a low-volatile organic modifier for the determination of cisplatin and its monohydrolyzed metabolite*" by Nygren, Y., Hemström, P., Åstot, C., Naredi, P., and Björn, E., J. Anal. At. Spectrom., 2008, 23, 948–954. Figure 2, 3, and Table 1 are reproduced by permission of The Royal Society of Chemistry.

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