Sanofi-aventis

Alnwick, Northumberland, UK

Success Story

A high throughput assay for oxaliplatin in clinical samples

An Agilent inductively coupled plasma mass spectrometer (ICP-MS) was selected as a replacement instrument for an under-performing ICP-MS at sanofi-aventis's Global Metabolism and Pharmacokinetics department in Alnwick, UK. In this publication, the department members Jonathan Scott, Mike Blackburn, Stuart McDougall and Peter White report on their experiences. The department's objectives were to establish suitable methodology in order to conduct fast, simple and reliable routine assays of oxaliplatin-derived platinum in plasma ultrafiltrate (PUF), plasma and urine. Initial method validation and comparative performance data with the lab's previous ICP-MS system is described. Further assay validations involving other platinum-based anti-cancer agents are also detailed.

Introduction

Oxaliplatin (eloxatin) is a novel platinum-containing anticancer (chemotherapy) agent which has been approved in the US and several major European countries for the treatment of adults with certain types of cancer including advanced colorectal cancer. Oxaliplatin undergoes rapid and extensive biotransformation, after intravenous administration, producing various active platinum-containing derivatives. Therefore, the ability to accurately quantify platinum in human biofluids is a prerequisite to an under-standing of their pharmacokinetics, pharmoco-dynamics and toxicity of the drug.

Within the department of Global Metabolism and Pharmacokinetics of sanofi-aventis, inductively coupled plasma mass-spectrometry (ICP-MS) is used to measure oxaliplatin-derived platinum. However, the limited sample throughput and matrix tolerance of the ICP-MS formerly in use at the facility prevented large-scale studies from being carried out in a timely manner. A project was undertaken to evaluate a replacement ICP-MS that would achieve the required increase in analytical productivity.

The Situation

The limited sample throughput and matrix tolerance of the ICP-MS equipment currently in use was preventing sanofi-aventis's Global Metabolism and Pharmacokinetics department from performing large scale studies on oxaliplatin-derived platinum. A project was initiated to evaluate replace-ment equipment that would achieve the required increase in analytical productivity.

The Solution

An Agilent 7500 ICP-MS instrument was chosen as the replacement equipment because of the excellent matrix tolerance, lower backgrounds, larger dynamic range and smaller footprint.

What is ICP-MS?

ICP-MS performs multi-elemental analysis with excellent sensitivity, ability to overcome matrix related interferences, sufficient linearity to measure a wide concentration range in unknown samples, and high sample throughput. It employs a plasma (ICP) as the ionization source and a mass spectrometer (MS) analyzer to detect the ions produced. It can measure measure most elements in the periodic table and determine analyte concentration down to the sub nanogram-per-liter (ng/L) or part-per trillion (ppt) level. It can perform qualitative, semiquantitative, and quantitative analysis, and since it employs a mass analyzer, it can also measure isotopic ratios. Figure 1 shows a schematic diagram of the Agilent 7500a ICP-MS instrument.

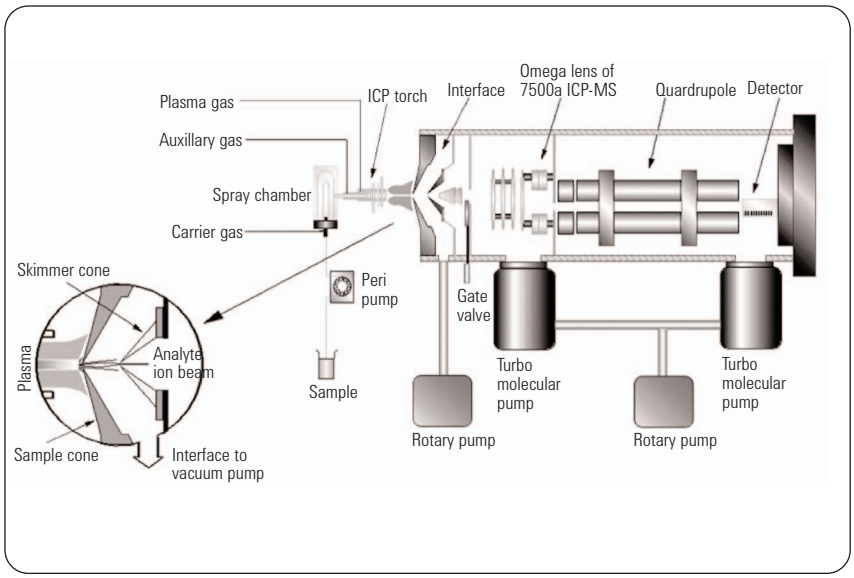

Figure 1
Schematic diagram of Agilent 7500a ICP-MS instrument.

Instrumentation

The Agilent 7500a was selected to support oxaliplatin analysis as it demonstrated the following features:

- Excellent matrix tolerance enabling high throughput runs with biological matrices
- Off-axis lens system resulting in lower backgrounds
- Dual mode detector to support a larger dynamic range
- Benchtop design to minimize use of laboratory space

The Agilent 7500a used throughout this study has equivalent performance to all 7500 Series instruments when operated in non-collision mode.

New sample assay methods

In order to maximize sample throughput in the laboratory, three new assays for plasma, plasma ultrafiltrate (PUF) and urine matrices were devised. Traditionally, all sample matrices had been prepared using a hazardous, one-hour, heated acid digestion. With the superior matrix tolerance of the Agilent 7500a, the prolonged digestion previously required was not necessary. It was found that dilution using tetramethyl ammonium hydroxide (TMAH), followed by 30 minutes mixing for plasma and urine, was effective.

Productivity was significantly increased due to the reduced sample preparation time and the analysis speed of the 7500a (4.5 min/sample compared to 8 min/sample with the previous instrument). Performance criteria of the original ICP-MS (old) and the 7500a are compared in tables 1 and 2.

Instrument	Old ICP-MS	Agilent 7500a
Dynamic range	1 – 100 ng/mL	1 – 1000 ng/mL
Sample volume	100 µL	100 µL
Preparation method	Add 2 mL of 1 % HNO_3	Add 900 µL of 1 % TMAH
Analysis time	8.5 minutes	4 minutes
Analysis capacity	70 samples	240 samples

Table 1
Comparison of ICP-MS performance for plasma ultrafiltrate assays.

Instrument	Old ICP-MS	Agilent 7500a
Dynamic range	300 – 10,000 ng/mL	100 – 10,000 ng/mL
Sample volume	100 µL	100 µL
Preparation method	Add 1 mL of HNO_3 digest for 1 hour at 90-100 °C and dilute to 10 mL	Add 9.9 mL of 1 % TMAH (30 minute mixing)
Analysis time	8.5 minutes	4 minutes
Analysis capacity	70 samples	240 samples

Table 2
Comparison ICP-MS performance for plasma assays.

Results

Table 3 gives a summary of the validation data generated using the 7500a for the analysis of various oxaliplatin-spiked PUF, plasma and urine samples.

Figure 2 shows the increase in oxaliplatin specimen analyses following the installation of the 7500a in 2000. This level of high throughput analysis would not have been possible with the original ICP-MS. The platinum assays have continued to perform in a reliable and robust manner.

Nominal (ng/mL)	n	Observed mean (ng/mL)	Mean % difference	
			Nominal	95 % CI
PUF 1.0	18	0.88	-12.0	-16.56, -7.19
PUF 2.5	18	2.39	-4.40	7.49, -1.33
PUF 100	18	103	3.00	0.36, 5.03
PUF 1000	18	980	-2.00	-3.51, 0.41
Plasma 100	18	95.3	-4.70	-7.12, -2.35
Plasma 250	18	238	-4.80	7.18, -2.55
Plasma 1000	18	970	-3.00	-6.10, 0.03
Plasma 10000	18	9480	-5.20	-8.41, 1.97
Urine 100	18	94.5	-5.50	-9.56, -1.42
Urine 250	18	240	-4.00	-7.33, -0.97
Urine 1000	18	1000	0.00	1.28, 1.74
Urine 25000	18	24300	-2.80	-4.72, 1.23

Table 2
Plasma ultrafiltrate, plasma and urine assay validation data.

Further assay validations

Additional platinum assays were subsequently developed and validated to support further clinical studies involving combinational therapy with other platinum-containing oncology agents. These assays were developed using the same sample preparation procedures and instrument methods described above. A summary of these is presented in table 4.

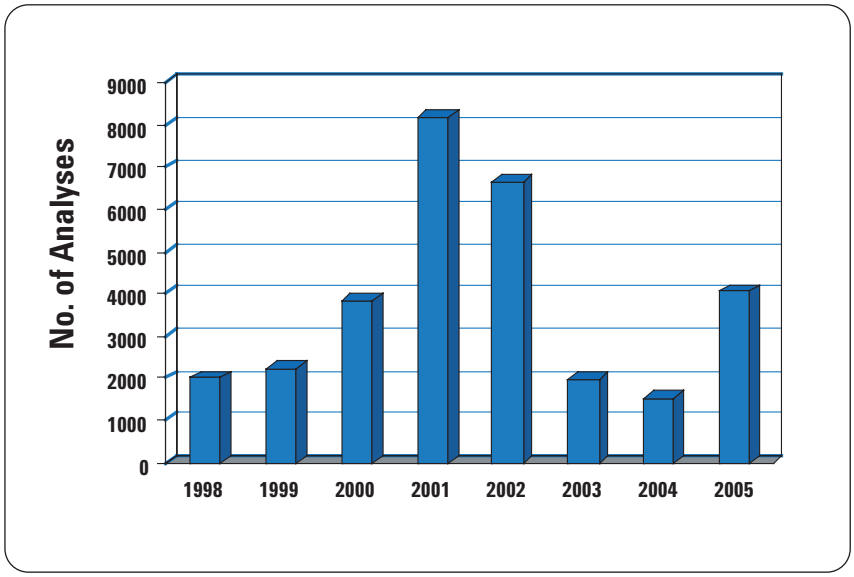

Figure 2.
Number of sample analyses by ICP-MS.

Compound	Cisplatin		Carboplatin
Matrix	PUF	Plasma	PUF
Dynamic range	1 – 1000 ng/mL	100 – 10000 ng/mL	1 – 1000 ng/mL
Sample volume	100 µL	100 µL	100 µL
Preparation method	Add 900 µL of 1 % TMAH	Add 9.9 mL of 1 % TMAH (30 minute mixing)	Add 900 µL of 1 % TMAH
Analysis time	4 minutes	4 minutes	4 minutes

Table 4
Summary of the additional assays developed.

Conclusions

Results obtained following deployment of the 7500a demonstrated that it was highly suitable to support the analysis of oxaliplatin-derived platinum undertaken at sanofi-aventis. Such analysis is vital in leading to an understanding of the pharmacokinetics, pharmacodynamics and toxicity of the drug. The department's investment in purchasing a new ICP-MS system was soon justified with rapid payback in terms of increased sample throughput of the assay procedures and improved reliability. Further assays, supporting analysis of specimens with different platinum-containing oncology agents have been developed, with minimum effort.

About Sanofi-aventis

Sanofi-aventis is one of the world's leading pharmaceutical companies and is number one in Europe. Its mission is to improve health throughout the world through innovative research and development and an international presence. Sanofi-aventis is focusing its R&D efforts on therapeutic areas which address major public health needs: thrombosis, cardiovascular and metabolic diseases, central nervous system disorders, oncology, internal medicine and vaccines.

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