An Elemental Shift in Radiopharmaceuticals

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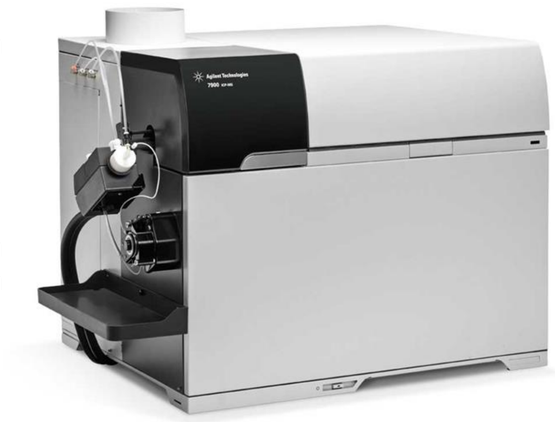
Background:
Radiotheranostics is a cutting-edge approach that integrates diagnostic imaging and targeted radiotherapy using radiopharmaceuticals to improve the precision and efficacy of disease treatment, particularly in oncology. This study explores the application of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) as a non-radioactive, cost-effective alternative for early-stage biodistribution studies of radiopharmaceuticals. By employing non-radioactive isotopes and optimising sample preparation protocols, the research demonstrates the feasibility of achieving single parts-per-million (ppm) detection limits across a range of medically relevant elements. The study also confirms the effective breakdown of DOTA chelates—commonly used in theranostics agents—through acid and microwave digestion, ensuring accurate elemental analysis. The optimised method achieved high spike recovery rates and maintained analytical precision even at sub-ppb concentrations. These findings support the use of ICP-MS as a viable tool for preclinical evaluation of radiopharmaceuticals, offering a safer and more economical alternative to traditional radioactive assays.

Biodistributiom studies are required for early stage Radiotheranostics in order to track:

- Distribution
- Persistence
- Clearance from the administration site

Measuring the concentration of the agent in various tissues over time can be expensive and time consuming leading to less options being taken through to distribution studies.



ICP-MS is a powerful analytical technique used to detect and quantify trace element isotopes in various sample types. It combines the capabilities of ICP for ionising the sample with mass spectrometry (MS) for detecting and measuring the ions produced.

Advantages:

- Superior detection to ppt levels
- High resolution, and robustness
- Lower costs and higher throughput

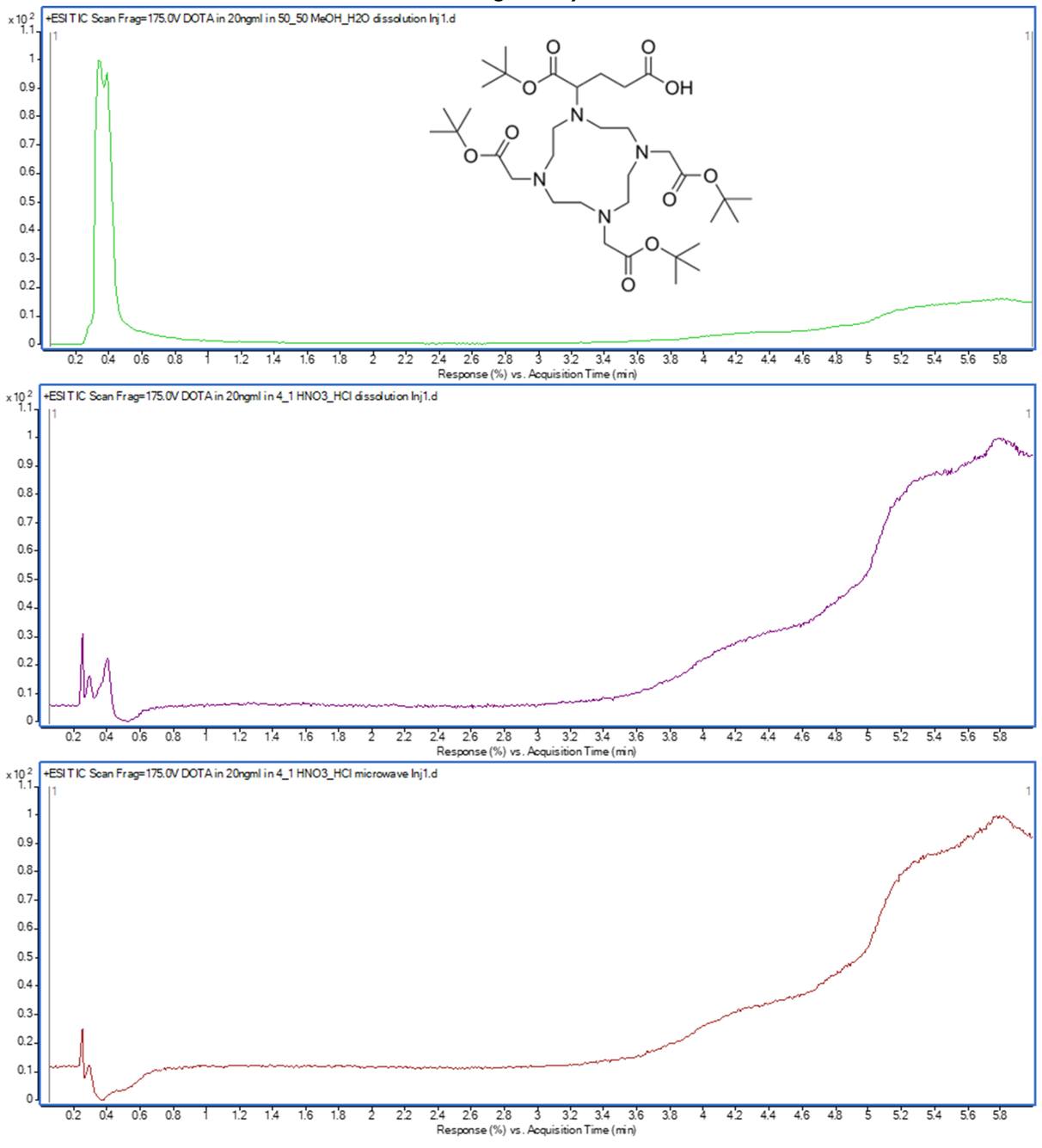
The solution - Swap hot-labelling for cold labelling analyse by ICP-MS!

Most theranostics studies utilise a DOTA chelator to bind the metal payload. Prior to analysis, it is essential to disrupt this complex, thereby releasing the metal for ionisation and subsequent detection. LC-MS was used to determine the presence of the DOTA-chelator after digestion.

Two samples were prepared for LC-MS analysis.

- The first underwent room temperature acid dissolution with 2 mL of concentrated nitric acid and 0.5 mL of concentrated hydrochloric acid added to approximately 5 mg of sample, followed by dilution to 50 mL with Ultra High Quality (UHQ) water.
- The second sample was digested using a MARS6 microwave system under high pressure and temperature in acidic conditions.
- The solutions were then diluted with UHQ water and then further diluted in a 50:50 MeOH/Water mixture to a final concentration of 20 µg/mL and sample analysis performed using an Agilent 1290 Infinity II UPLC system with Agilent 6230 ToF Mass Spectrometer.

Figure 1: Top: DOTA-GA (tBu)4 20 ng/mL dissolved in 50:50 Methanol/Water (no digestion), Middle: DOTA-GA (tBu)4 20 ng/mL dissolved in 50:50 Methanol/Water (Room temperature acid digestion), Bottom: DOTA-GA (tBu)4 20 ng/mL dissolved in 50:50 Methanol/Water (microwave digestion)



The room temperature digestion showed the digestion reaction had nearly fully destroyed the cage and the microwave digested sample showed no trace of the DOTA-GA (tBu)4 peak indicating the DOTA cage was completely destroyed.

Although we had complete destruction of the DOTA cage by microwave digestion, room temperature digestion was chosen for subsequent experiments as significant dilution is necessary after microwave digestion. Room temperature digestion allows us to have much lower dilution factors and therefore LOQs. For method optimisation elements similar to those currently used for radiolabelling were assessed: Copper (Cu), Gallium (Ga), Yttrium (Y,) Rhodium (Rh), Indium (In), Lanthanum (La), Terbium (Tb), Lutetium (Lu), and Lead (Pb).

Samples were prepared using lower acid concentrations, ranging from 10% (v/v) to 5% (v/v) total acid and final solution volumes (50 mL to 5 mL), to reduce the dilution factor. All resulting solutions were transparent and deemed suitable for analysis by ICP-MS using an Agilent 7900.

A variation of diluents were assessed to allow for the digestion of different peptide types. The diluents that provided acceptable digestion of the DOTA chelator without impeding the linearity of the elements are detailed in **Figure 2**.

Figure 2: An example of the comparison of the effects of diluents capable of breaking the DOTA chelator cage on the linearity of the elements

Spike recoveries for all elements were within the pharmacopeia guidelines acceptance criteria of 90-110%, confirming that a final sample volume of 5 mL is appropriate for analysis. The sample preparation process demonstrated that dissolving 5 mg of sample to a total volume of 5 mL was appropriate for analytical purposes.

This approach produced a dilution factor of 1000, whereby a solution concentration of 0.001 ppb corresponds to 0.001 ppm in the original sample. A DOTA-GA (tBu)4 sample spiked in triplicate with elemental concentrations ranging from 0.01 ppb in solution (0.01 ppm in the sample) to 0.25 ppb in solution (0.25 ppm in the sample) were assessed to ensure accuracy at the lower regions of the method range, refer to **Table 1**. The results show that recovery remains accurate and precise even at sub-ppb concentrations of a complex matrix sample.

Table 1: Table detailing the regression of each elemental isotope assessed and the recoveries at 0.01 ppb

Element / Isotope	Correlation Coefficient (r)	Spike Recovery range %
63 Cu	0.9999	101.6 % ±5.1
71 Ga	0.9999	103.0 % ±3.2
89 Y	0.9998	97.9 % ±1.4
103 Rh	0.9997	98.2 % ±1.1
115 In	0.9998	98.6 % ±1.2
139 La	0.9996	98.2 % ±1.3
159 Tb	0.9995	98.9 % ±2.5
175 Lu	0.9995	98.7 % ±2.3
208 Pb	0.9994	97.1 % ±0.9

Conclusion:

This study highlights the promising potential of ICP-MS alternative for early-stage biodistribution studies in Radiotheranostics:

- Sub-ppm quantification limits with complex matrices to 0.001 ppm
- Simple and effective method for the successful degradation of DOTA chelates.
- Highly accurate and repeatable at low sensitivities with a range of readily available element options