Journal Name

ARTICLE

Quantitation of the Fe spatial distribution in biological tissue by on-line double isotope dilution analysis with LA-ICP-MS: A strategy for estimating measurement uncertainty

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A novel strategy is reported for the quantitative analysis of the Fe spatial distribution in biological tissue using laser ablation with ICP-MS and on-line double isotope dilution analysis (LA-ICP-IDMS). The proposed on-line IDMS method involves post-ablation introduction of an isotopically enriched ^{57}Fe spike solution using a total consumption nebuliser. To investigate the potential applicability of the developed method to biological tissue with varying Fe concentrations (akin to those observed in bio-imaging), the effect of sample-to-calibration standard blend ratio on the accuracy of the Fe data was investigated over a range of 1:0.2 to 1:10. To achieve this, homogenised sheep brain tissue doped with Fe (251 $\mu g \cdot g^{-1}$) was used as the model sample. Recoveries of 80–109% of the expected Fe concentration in the model tissue sample (as determined by ID-ICP-MS of the tissue digest) were obtained over a sample-to-standard ratio range of 1:1 to 1:5. A systematic estimation of measurement uncertainty for LA-ICP-IDMS was undertaken and for the first time the mass flow rate of the material was determined *via* single-IDMS. An overall combined expanded uncertainty (k = 2) of 15 –27% was achieved for ratio matching of 1:1 to 1:5. The factors with greatest contribution to the overall uncertainty were the mass of spike, the measured ratio of the standard blend and the mass of calibrant. External calibration with internal standardisation was perfomed on the same model sample for the purpose of comparison. The measurement uncertainty associated with this calibration approach was for the first time estimated for LA bio-imaging by taking into account the contributions from the signal intensity variance, the errors from least squares regression and concentration of the standards. For external calibration the overall relative expanded uncertainty was approximately 50% (k = 2), with the uncertainty in the linear least squares regression (R^2 of 0.9833) and the signal variation being the main contributing factors. The results for Fe in the model sample agreed well with those determined *via* LA-ICP-IDMS. For the first time, the potential of a LA-ICP-MS isotope dilution calibration strategy to validate higher throughput calibration methodologies (e.g. matrix-matched external calibration with internal standardisation), as would be required for routine medical applications, has been demonstrated.

Supplementary Material

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Table S1. Operating conditions for Agilent 7700x ICP-MS

ICP-MS (Agilent 7700x)	
Plasma RF power	1550 W
Nebuliser	MicroMist quartz concentric
ICP cones	Ni
Spray chamber	Scott-type double pass (2°C)
Cooling gas flow	15 L·min^{-1} Ar
Carrier gas flow	1.05 L·min^{-1} Ar
Hydrogen gas flow	4.3 mL·min^{-1}
Isotopes monitored	^{56}Fe, ^{57}Fe
Integration time/mass	90 ms
Peak pattern	3 points
Replicates	10
Sweeps/replicate	100

Characterisation of total Fe tissue concentration by digestion and double-IDMS

Operating parameters for the determination of total Fe concentration in doped tissue are shown in Table S1.