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K d : Bovine bone; Autoclave sterilization; Lyophilisation; LA-ICP-MS

I t d ct

e atomic distribution and concentration of bene cial or toxic metals and non-metals in biological tissues is of great interest. Over the past decade, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) has become as a powerful analytical tool for the purposes of qualitative imaging or mapping of element concentrations [1]. LA-ICP-MS can depict analytic distributions in biological tissue sections with a spatial resolution ranging from 10-100 µm [2] and o ers a detection limit from parts per million to as low as parts per billion [3]. ese are bene ts that make LA-ICP-MS a versatile tool in mapping the elements making up so and hard tissues, thereby contributing better understanding of biological processes especially where the quanti cations of these trace elements are concerned [1,4,5].

During the past decade LA-ICP-MS has been applied to a number of applications to investigate biological structures from ranging from human and animal tissues [6,7], plants tissues [2] and other biological samples such as hair [8] or synovial uid samples [9]. In human tissues this method has been used with biopsy sections from breast [10], lymph node [11], brain [12] and eye lenses [13], and of particular relevance for the present study, ICP-MS analyses have been reported for mineralised tissues [14-16].

For quantitative analyses with LA-ICP-MS, reproducibility at 5% and better can be achieved if two conditions are satis ed: 1) an internal standard element can be quanti ed or estimated accurately, and 2) a matrix-matched Certi ed Reference Material (CRM), is available. e reasons that these conditions are necessary related to the fact that all

Citation:

Methods	Sample	^{24}Mg ppm	^{29}Si ppm	^{44}						

groups, suggesting that these elements were not homogenously distributed in the natural bone fragments.

As for ^{59}Co, the concentration was higher in non-autoclaved bone samples compared to autoclaved bone samples, suggesting an e ect from autoclaving that results in greater variability of the ^{59}Co distribution within samples and variability in ICP-MS measurement. As a whole we judged both procedures as equally suitable for the preparation of bone standards. e non-autoclaved bone pellets were marginally better as a matrix-matched reference material for mineralised tissues by being more economic and faster to manufacture.

I d t a a -a t c a d b d

e elemental concentrations of magnesium, calcium, iron, silicon cobalt, strontium and lead released from non-autoclaved bone powders were shown in Table 2. When compared to the data from ICP-AES, it demonstrated that ICP-MS is a more powerful tool which is sensitive with lower limit for some of the elements, such as Si, Ti and Pb. On the other hand, ICP-AES showed the higher concentration for Ca, Zn and Sr when compared to that of ICP-MS data.

LA-ICP-MS a t at a b a t

A biogenic signal is expected to produce a constant concentration of the elemental composition of a given region and is therefore an important parameter to assess natural bone fragments. Cross-sections of seven replicates were analysed to obtain concentration pro les of elements such as Mg, Si, Ti, and Fe (Figure 1 and Table 2). Regions 1-7 from Figure 1 exhibited large variation in the concentration of all the elements studied compared to their mean value, suggesting an unstable signal in these bone fragments. Similar results were observed in the other four bone samples analysed under the same conditions by LA-ICP-MS. e data shown in Table 2 reveals a degree of variation in the signal of all the elements as well as a signi cantly higher standard deviation resulting from seven replicates, and suggests that the elements in natural bone fragments are not homogenously distributed. Natural bone fragments are therefore of limited utility as internal standards.

H a a -a t c a d b t

e homogeneity of non-autoclaved bone samples (n=4 donors, 10 replicates for each donor) were analysed by LA-ICP-MS for elemental distribution of C, Na, Mg, K, Ca, Mn, Fe, Zn, Sr, Hg and Pb (Figure 2). e signal intensity exhibited only a minimal standard deviation across the replicates and suggests that lyophilisation is the optimum way of drying the samples prior to ICP-MS analyses since it appears not to result in any loss of elements.

A further test of homogeneity of non-autoclaved bone pellets was shown with macroscopic and light microscopy images, which demonstrated the particle size was less than 100 um and distributed evenly on the surface. A er normalization of non-autoclaved bone pellets against SRMs, the data from 37 replicates for each donor (4 donors) show that the individual scatter plots cluster close to their mean value with high precision for all the elements (Figure 3).

e lyophilisation technique described here is an ideal method for quantitative assessment of any mineralised tissues including teeth and bones and only requires a timeframe of approximately 24 h using a single standard. With such a matrix-matched standard, LA-ICP-MS methods can be applied in the study of mineralized tissues with metal isotopes revealing not only the intensity but also the sources of metal exposures in at-risk communities. However, the feasibility of its application as suitable standard require further evaluation by analysing and comparing the results of a real bone or tooth sample analysis from using NIST SRM 610/SRM 612 and the non-autoclaved bovine bone pellets.

C c

In this study, non-autoclaved bone pellets were shown to be suitable as internal standards for quanti cation experiments of samples with similar biological properties, such as bovine tibial bone. We showed that this standard yielded high accuracy of elemental quanti cation and was simple and fast to produce. Freeze-dried bovine bone pellets as standards are matrix-matched reference materials that are reliable, exact and quantitative, and therefore suitable for a wide range of elements.

Acknowledgements

The work was supported by the facilities at the Institute of Health and Biomedical Innovation (IHBI) and the Institute for Future Environments (IFE) of Queensland University of Technology. The authors would like to thank the assistance from Mr. Edward Ren and IFE staff during the LA-ICP-MS investigations and data analysis.

Citation: Han P, Zhou Y, Lu S, Lloyd T, Friis T, et al. (2015) Utilisation of Bovine Bone Pellet as a Matrix-Matched Reference Material for Calcified Tissues in LA-ICP-MS Application. J Anal Bioanal Tech S13: 006. doi:10.4172/2155-9872.S13-006

21. Vasinova Galiova M, Nyvltova Fisakova M, Kynicky J, Prokes L, Neff H, et al. (2013) Elemental mapping in fossil tooth root section of Ursus arctos by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Talanta 105: 235-243.

22. Hellstrom J (2008) Iolite: software for spatially resolved LA-(quad and MC)-ICP-MS analysis. Mineral Association of Canada.