Application Note

intelligent sample preparation

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ePrep Sample Preparation Workstation | Application Note 2019

Calibration Standards preparation at ultra trace levels (ppt) for ICP-MS elemental Nanoparticle analysis

INTRODUCTION

Biomedical imaging is one of the driving forces in modern healthcare research. It allows non-invasive investigations of morphological, anatomical, and physiological changes in patients. Contrast agents are materials with the ability to enhance contrast commonly administered before the imaging process and have an important function for early diagnosis

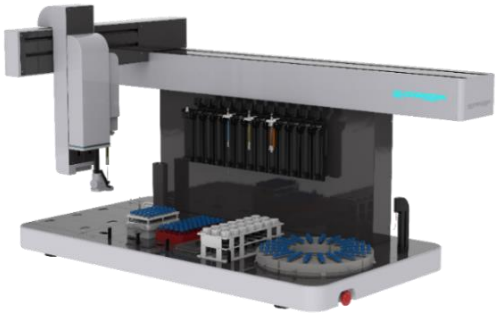

Figure 1. ePrep Sample Preparation Workstation

Nanoparticles (NPs) are under investigation as novel contrast agents and promise improved performance in diagnostic imaging. Accordingly, current advances in nanotechnology may lead to a NP-based dedicated contrast agent that may provide enhanced sensitivity and specificity in imaging enabling and therefore, earlier diagnosis [1].

Nanoparticles carrying Gd^{3+} ions are particularly interesting as they have a large paramagnetic momentum which polarises protons in water enhancing signal, significantly. The design of such particles require dedicated analytical methods which allow a precise and accurate elemental determination, while providing high sensitivity, a large dynamic range and robustness.

In this context, the ePrep workstation (Figure 1) is used as a facile and rapid solution to prepare samples and calibration standards for ICP-MS to quantify various elements in synthesised nanoparticles. The ePrep workstation automates and thus, facilitates and accelerates sample preparation while providing high accuracy and precision.

Manual preparation of these samples and standards requires a certain level of experience and skill, as it is important that factor influencing precision and accuracy are understood and addressed by the analyst in order to achieve a suitable level of performance meeting laboratory standards. Some sources of error in manual sample preparation can be addressed by careful and thoughtful method design, however, the performance of lab personal is often diminished through tedious and repetitive tasks that must be done daily and at a high quality.

This work compares the preparation of ICP-MS calibration standards conducted by ePrep workstation operated by a first-time user (a) against standards prepared manually by (b) a scientist trained in analytical chemistry and by (c) a scientist new to analytical chemistry.

The calibration protocol for the preparation of calibration standards containing a set of seven solutions ranging from 0.1 to 500 ng/g (Na, K, Y, Gd, Er, Tm and Yb) and 2 internal standards at 25 ng/g (In, Tl) is described in Figure 2. The prepared solutions were analysed employing a single quadrupole ICP-MS (iCAP RQ, Thermo Fisher Scientific, Australia) equipped with an ASX560 auto sampler (Teledyne CETAC, Omaha, NE, USA).

PROCEDURE

ePrep Automated Standards Preparation:

The ePrep Sample Preparation Workstation enabled the automated preparation of:

A: Several calibration standards ranging from 0.1 to 500 ng/g and containing 25 ng/g internal standard;

B: Blank solution spiked with 25 ng/g internal standards

The standard stock solutions were at 1000 ng/g and 100 ng/g for each element of interest and a third stock solution contained the internal standards at 5000 ng/g.

The protocol incorporates syringe rinsing steps to eliminate carryover, and multiple mixing steps for homogenous preparation of standards.

ePrep Process:

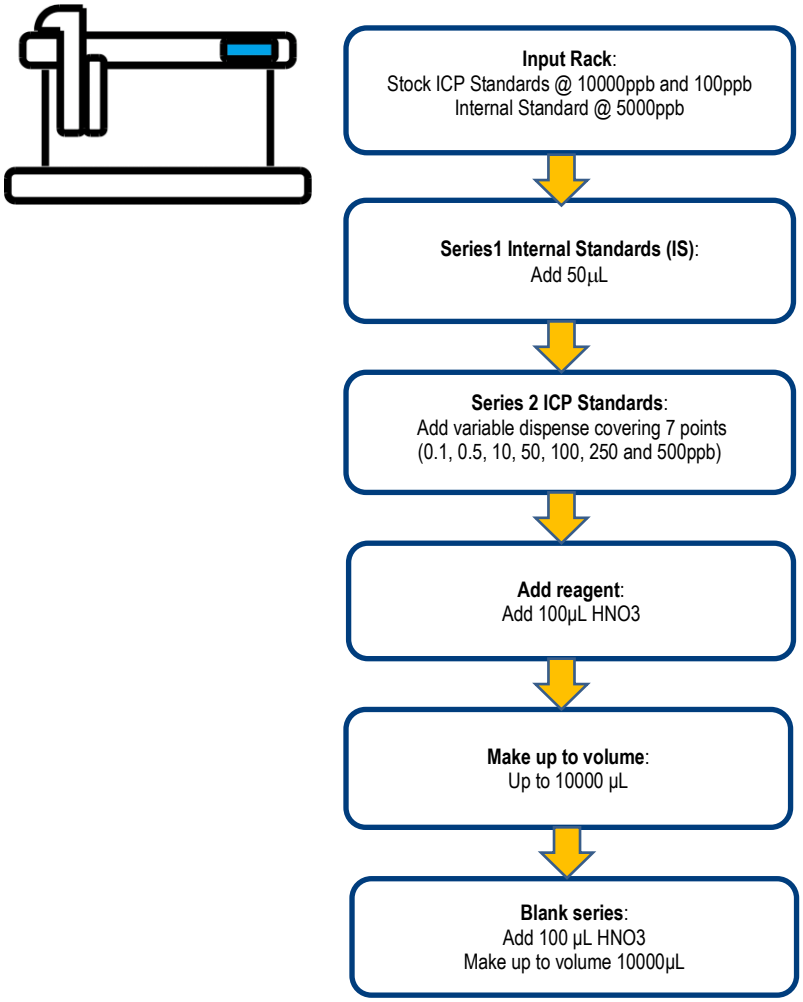

Figure 2: ePrep Automated Calibration Standard Workflow

Manual Standards Preparation:

Manual sample preparation followed the same User steps as the ePrep procedure. Pipettes are gravimetrically calibrated weekly to ensure accuracy. Pipette calibration is a 25min procedure.

RESULTS

The automatically prepared standards were compared to standards prepared by an experienced and unexperienced analyst, respectively, who used standard lab equipment. Standards were analysed threefold and the average signal was plotted against concentration as depicted exemplary in Figure 3 for ^{172}Yb and ^{156}Gd. Pearson's R^2 values are often used as a measure of linearity of resulting fitted calibration curves and curves with a low R^2 value may impact accuracy of the analysis.

The Pearson's R^2 values were determined for data sets obtained from automated preparation employing the ePrep workstation as well as from the two manual preparations. The raw intensities were normalised to the internal standard signals to allow comparison between data sets obtained at different dates and different tune conditions. The R^2 values are listed in Table 1 and compared between data sets. It can be seen that the ePrep workstation achieved values higher than 0.999 in all cases. The quality of the automated standard preparation was comparable to calibration outcome by the experienced and skilled analyst achieving similar sensitivities and comparable linearity.

The preparation for an eight-point calibration curve containing two internal standards including acidification required 90 minutes for the unexperienced analyst, 40 minutes for the experienced analyst and the ePrep workstation required 20 minutes.

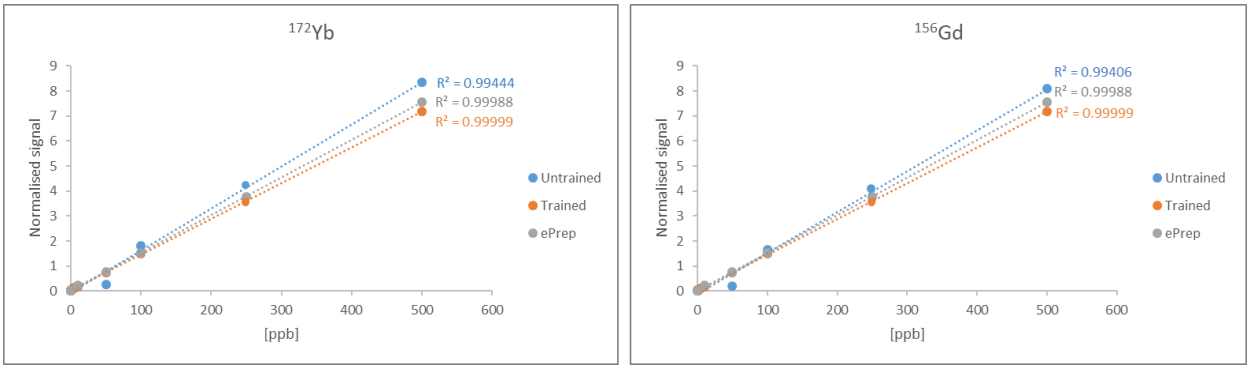

Figure 3: Representative calibration curves for 172Yb and 156Gd analyses by ICP-MS.

Table 1 Linearity (R^2) for each of the elements prepared using ePrep vs manual preparation by an experienced vs unexperienced Analyst. ePrep results indicate they are comparable with the Experienced Analyst and are superior to the Inexperienced Analyst.

	R² Linearity		
Element	**ePrep (automated Preparation)**	**Experienced Analyst (manual preparation)**	**Unexperienced Analyst (manual preparation)**
Na	0.99993	0.99977	0.99139
K	0.99901	0.99993	0.99370
Y	0.99976	0.99993	0.99357
Gd	0.99988	0.99999	0.99406
Er	0.99997	0.99999	0.99349
Tm	0.99999	0.99999	0.99310
Yb	0.99988	0.99999	0.99444

CONCLUSION

Testing of synthesised nanoparticles for elemental analysis requires almost daily preparation of external calibration standards. This is a laborious task and requires a high level of expertise for the required precision and accuracy. Results can vary dramatically depending on user competence.

The ePrep Sample Preparation Workstation was employed by a first-time-user for preparation of external calibration standards for trace metal ICP-MS analysis. Seven concentrations and a blank solution were prepared, acidified and spiked with two internal standards within 20 minutes. The preparation was fully automated on the ePrep which was significantly faster than those prepared manually by two analysts. The performance of the ePrep-based calibration was comparable to a skilled experienced analyst.

The ePrep can be programmed and operated by unskilled laboratory staff for the preparation of samples and external standards for analytical analysis. This significantly reduces analysis cost and time.

ACKNOWLEDGEMENTS

Gonzalez de Vega, R[1]; Clases, D[1]; Du, Z. [1]; Xu, H[1]; Doble, P[1]
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2) Potential Sources of Analytical Bias and Error in Selected Trace Element Data-Quality, Scientific Investigations Report 2016-5135 (https://pubs.er.usgs.gov/publication/sir20165135); Angela P. Paul, John R. Garbarino, Lisa D. Olsen, Michael R. Rosen, Christopher A. Mebane, and Tedmund M. Struzeski

3) Quality of the Trace Element Analysis: Sample Preparation Steps; (http://cdn.intechweb.org/pdfs/23729.pdf); Maja Welna, Anna Szymczycha-Madeja and Pawel Pohl

ePrep WORKFLOW

Stock solutions: 1000ppb and 100ppb (Initial volume 25mL)
Internal standard (IS): 5000ppb (initial volume 25mL)
Create a 7 points calibration curve (0.1, 0.5, 10, 50, 100, 250 and 500ppb)

WORKFLOW Internal Standard

a) Add Internal Standard: 50µL (tubes 1-8)

WORKFLOW WORKING STANDARDS

a) Variable dispense:
   From stock solution 100ppb (tubes 2-5): 2, 10, 200, 1000µL
   From stock solution 1000ppb (tubes 6-8): 1000, 2500, 5000µL
b) Add regent: 100µL HNO3
c) Make up to volume: 10000µL with Ultra-pure water

WORKFLOW BLANK

d) Add reagent: 100µL HNO3
e) Make up to volume: 10000µL with Ultra-pure water
f) Aspirate and dispense flows and Needle depth: Auto