ICP-MS: A Candidate Reference Method for Sweat Chloride Quantification

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Background

The sweat chloride test has been an essential tool for the investigation of Cystic Fibrosis since a reliable test was described in 1959. Even since the beginning of the genomic era the sweat test remains a crucial diagnostic assessment to determine disease severity, Figure 1. Current acceptable methods for chloride analysis, suggested by evidence based guidelines, are usually derived from higher concentration range serum based methods. Some of these require an addition dilution approach to achieve the analytical sensitivity required for sweat. A matrix matched JCTLM reference method for sweat chloride is yet to be identified. With the use of the high sensitivity testing of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) we proposed to develop a gold standard method for measuring sweat chloride including a comparison study of on-line and off-line internal standard introduction.

Methods

20 ul aliquots of standards, quality controls and volunteer samples (collected by Wescor method), were prepared simultaneously in ICP-MS tubes with 20 ul of gallium as the off board internal standard and 1.96 ml of 1% v/v Ammonium diluent. ICP-MS analysis was performed on an Agilent Technologies 7700x ICP-MS equipped with an Agilent Technologies ASX 520 Auto sampler, Figure 2. Argon was used as the gas carrier with a plasma flow of 15.0 L/min and Helium gas was used in the collision chamber with a flow of 1.0 L/min. A five point calibration curve, including blank, was established with the lowest standard at 5.6 mmol/L (2000ppb), Figure 3. An ICP-MS Internal Standard Mix was included where Germanium and Scandium were selected as the on-line IS. Independent evaluation of performance was determined through participation in the RCPAQAP Sweat Testing program throughout 2013.

Results

The method demonstrated sensitivity (LOQ) to <1 mmol/L with the analytical range up to at least 200 mmol/L. Between run imprecision and bias were assessed during RCPAQAP cycle 28 against weighed in target values at six values from 10 to 98 mmol/L. Results demonstrated imprecision of <2% and a bias of <1 mmol/L across the concentration range. A comparison between the on-line and off-line IS methods demonstrated a clear increase in precision and accuracy from the off-line IS, Figure 4.

Conclusions

With the use of the high sensitivity testing of the ICP-MS a potential gold standard method for measuring sweat chloride has been developed. This technique exhibits a superior method to those described in the guidelines because of the demonstrated linearity, stability, reproducibility and functionality. This project succeeded in its primary aim of developing a candidate reference method for the analysis of sweat chloride.

References


Figure 1: Common genetic defects related to the transport of chloride in the sweat gland associated with varying severity of Cystic Fibrosis. This is the fundamental basis for the mechanisms behind the sweat collection methods.

Figure 2: Agilent 7700x ICP-MS with ASX 520 Auto sampler

Figure 3: Agilent Mass Hunter for ICP-MS: ISTD Stability Graph of on-line and off-line internal standards, Calibration Curve and analyte quantitation windows.