Validation of a Bioanalytical ICP – MS Method for Quantification of Potassium in Human Urine

Synopsis

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is being increasingly utilised to quantify the concentration of elements in biomatrices in support of non-clinical and clinical studies. These data support pharmacokinetic (PK), toxicokinetic (TK), and bioequivalency (BE) evaluations. The purpose of this paper is to present a bioanalytical ICP-MS method validation procedure applicable to the quantification of potassium in human urine in support of BE studies. This method was validated following applicable bioanalytical guidance documents, and includes assessments of sensitivity, selectivity, linearity, carryover, intra/inter-day accuracy and precision, matrix effect, ruggedness, and various stability parameters. The method objectives, materials, results, and conclusions are presented.

Keywords

Inductively Coupled Plasma Mass Spectrometry, ICP-MS, Good Laboratory Practice, Clinical, Bioequivalence, Bioanalytical Method, Method Validation, Potassium, Human Urine

Introduction

Potassium chloride is used to treat patients with hypokalemia and/or prevent hypokalemia for patients who are at risk of developing hypokalemia. Potassium chloride has been approved by the United States Food and Drug Administration (US FDA) as a treatment for hypokalemia. A non-binding draft guidance on potassium chloride as recommended by the FDA was issued in 2011 for drug submission purposes[1]. The objective of this study was to validate a bioanalytical method for the quantitation of potassium in human urine samples obtained from clinical studies with patients dosed with potassium chloride in support of required bioequivalence studies.

Current methods for the determination of potassium in biological fluids include atomic absorption spectrophotometry[2], ion-selective electrode[3], and inductively coupled plasma optical emission spectrophotometry[4]. These methods were used to support health screening purposes and were not intended for application to bioequivalence studies which require that the method be validated in compliance with requirements set forth by regulatory authorities such as the FDA or the European Medicine Agency (EMA).

ICP-MS is a relatively new technique that has been widely applied for sensitive, accurate, and efficient quantitation of elements in environmental samples, drug articles, and biological samples. Herein we present a bioanalytical ICP-MS method for the determination of potassium in human urine. This method was validated in accordance with the US FDA Guidance for Industry for Bioanalytical Method Validation[5], 21 Code of Federal Regulations (CFR) Part 58 GLP regulations[6], industry conference bioanalytical method validation meeting reports[7-10], and current MPI Research standard operating procedures (SOPs). Our approach was to apply the bioanalytical acceptance criteria and study design, typically regulated by the FDA bioanalytical method validation guidance document[5], to the ICP-MS analysis of potassium using a platform that is historically applied to environmental analyses.

Materials and Methods

Standards and Reagents:

Potassium chloride standard reference material (SRM 999b) was obtained from the National Institute of Standards and Technology (NIST) and was utilised for the preparation of all quality control (QC) samples. NIST traceable aqueous potassium and scandium standards (10,000 µg/mL) were obtained from Ultra Scientific and used for the preparation of calibration standards and the internal standard, respectively.

Ultrex, ultra-pure grade, nitric acid (HNO3) was obtained from J.T. Baker (Mallinckrodt Baker, Inc., Phillipsburg, New Jersey). Reagent water with a resistivity ≥18 MΩcm was obtained from a NanopureTM RO reversed osmosis system (Barnstead International, Dubuque, Iowa).

Control Matrix:

Control matrices used for the preparation of QC samples (Bioreclamation, Inc., Westbury, New York) were prescreened for endogenous potassium concentrations. The control matrix with the lowest endogenous potassium concentration among screened lots was used to prepare QC samples.

Replicates of the blank control matrix were analysed in every run to provide a baseline for subtraction from the fortified QC samples prior to determining the percent relative error.

Preparation of Solutions and Samples:

Internal Standard Working Solution (ISWS) and Calibration Stock Solutions

The 10 µg/mL ISWS and 100 µg/mL potassium calibration stock solutions were prepared by dilution of the scandium and potassium standards in 1% HNO3 (diluent).

Calibration Standards

With consideration to endogenous concentrations of potassium in human urine, calibration standards ranging from 500 to 10,000 µg/L were prepared in 1% HNO3 (diluent). The instrument

concentrations in diluent standards are equivalent to 100 to 2,000 µg/mL relative to urine when multiplied by a dilution factor of 200, as reflected in the sample preparation steps.

QC Fortification Solution (100,000 µg/mL)

The QC fortification solution was prepared by dissolving potassium chloride reference standard (NIST SRM 999b) in reagent water.

QC Samples

QC samples at the low, mid, high, and dilution QC concentrations were prepared in unfiltered human urine. As calibration standard solutions were made in diluent, additional diluent QC samples were prepared to verify the validity of the calibration curve throughout an analytical run, and, more importantly, the ability of the curve to accurately quantify the analyte in both matrix and diluent QC samples. The concentrations of matrix and diluent QC samples are presented in Table 1.

Sample Preparation Procedure:

Table 1 QC Samples

Matrix	QC Sample	K Concentration (µg/mL)
Urine	QC Low	200
	QC Mid	400
	QC High	600
	QC Dilution	4000
Diluent	QC Low	200
	QC Mid	800
	QC High	1200

Standards, QCs, blank matrix, and unknown study samples were thawed unassisted, gently mixed by inversion, and the solids were then allowed to settle to the bottom of the tube. A 50 µL aliquot of the supernatant was transferred to a clean 15 mL tube. A 100 µL aliquot of ISWS and 9.85 mL of diluent was added to each tube. The tubes were capped, mixed, and an aliquot was analysed by ICP-MS.

Equipment and ICP-MS Instrument Settings:

A Thermo-Finnigan ELEMENT2 sector field ICP-MS and a Perkin-Elmer Elan 6000 quadrupole ICP-MS were used. Typical instrument parameters are presented in Table 2.

Table 2 ICP-MS Instrument Settings

Instrument Parameters	Thermo Element 2 Sector Field ICP-MS (High Resolution Mode)	Perkin Elmer Elan 6000 ICP-MS
Wash Time:	60 seconds	60 seconds, 48 rpm
Sample Rinse Time:	40 seconds	40 seconds, 48 rpm
Sample Uptake Time:	20 seconds	20 seconds, 24 rpm
Analysis Time:	10 seconds	10 seconds, 24 rpm
Radio Frequency (RF) Power	1.3 KW	1.0 KW
Argon (Ar) cool gas flow	15.0	15
Ar Auxiliary gas flow	0.75	0.70
Nebuliser gas flow	0.95	0.89
Nebuliser	PFA-100	Cross-flow
Spray Chamber	Cyclonic	Double pass

Results and Discussion

System Suitability Test:

The instrument was tuned to optimise performance prior to each run. The typical evaluated performance parameters included: sensitivity, resolution, stability, oxide, and doubly-charged ion ratios. The specifications were listed in Table 3.

Table 3 System Suitability Test Parameters

Evaluation Parameters	Thermo Element 2 sector field ICP-MS	Perkin Elmer Elan 6000 ICP-MS
Sensitivity	> 1,000,000 cps (per 1 µg/L ^{115}In at low resolution mode)	> 200,000 cps (per 10 µg/L ^{103}Rh)
Resolution	> 9,000 (M/ΔM, at high resolution mode)	0.7 +/- 0.05 (10% Peak height)
Doubly Charged ratio	< 0.05% ($^{138}Ba^{2+}/^{138}Ba$)	< 0.05% ($^{137}Ba^{2+}/^{137}Ba$)
Oxide level	< 0.05% (CeO/Ce)	< 0.005 (BaO/Ba)
Signal Stability, %RSD	< 3%	< 3%

Selectivity:

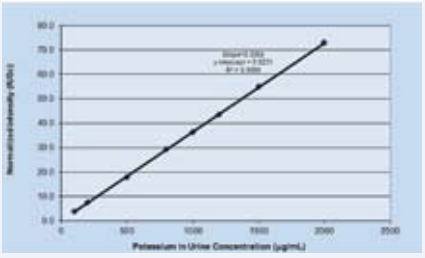
Selectivity was evaluated in six individual lots. Because of the endogenous nature of the analyte, whose concentrations vary across the entire calibration range among samples collected from the normal population, it is impossible to receive analyte-free blank urine for the evaluation of method selectivity. The analyte responses were reported as observed with no acceptance criterion. However, the scandium responses in these lots were compared to that obtained with a prepared calibration blank. The scandium intensity in all six lots did not exceed 5% of the signal intensity of the calibration blank.

Linearity:

The calibration standards were analysed in duplicate with one curve each at the beginning and the end of each run. A linear regression with no weighting was used to calibrate the instrument response.

The calibration curves had coefficients of variance (R2) of ≥0.998, which met the acceptance criterion (R2 ≥0.995). Figure 1 shows a representative calibration curve in the validation run.

Figure 1 Representative Calibration Curve



Carryover:

Carryover was assessed by injection of one reagent double blank after each upper limit of quantitation (ULOQ) standard in each analytical run. The analyte and IS responses in the reagent double blanks met the

acceptance criteria that these should be below 20% and 5% of the respective responses in the preceding lowest limit of quantitation (LLOQ) sample.

LLOQ:

An assessment of the accuracy and precision at the LLOQ was conducted in two analytical runs (n=6 each). The intra-day accuracy results were 8.4% and 1.9% relative error (RE), and the intra-day precision results were 1.3 % and 0.4% relative standard deviation (RSD), respectively. Both met the ±20% RE and ≤20%RSD acceptance criteria.

Accuracy and Precision:

The intra day accuracy of the QC samples (n=6) was determined for each core validation run. The inter day accuracy of the matrix QC samples was determined across the three core validation runs. The acceptance criterion was a mean concentration within ±15.0% RE of the nominal concentration for each level. For the QC low samples, the intra day accuracy for the three core validation runs ranged from 10.1% to 6.0%, and the inter day accuracy was 0.9%. For the QC mid samples, the intra day accuracy ranged from 5.8% to 1.3%, and the inter day accuracy was 3.1%. For the QC high samples, the intra day accuracy ranged from 5.4% to 2.6%, and the inter day accuracy was 0.1%. All results met the acceptance criterion.

The intra day precision of the QC samples (n=6) was determined for each validation run. The inter day precision of the QC samples was determined across the three core validation runs. The acceptance criterion was a RSD ≤15.0% for each level. For the QC low samples, the intra-day precision ranged from 1.2% to 3.4%, and the inter day precision was 4.3%. For the QC mid samples, the intra day precision ranged from 0.54% to 2.1%, and the inter day precision was 3.5%. For the QC high samples, the intra day precision ranged from 0.74% to 3.5%, and the inter day precision was 3.8%. All results met the acceptance criterion.
Results from diluent QC samples in the three core validation runs also met the acceptance criteria (RE ±15.0%, RSD ≤15.0%). The intra-day accuracy ranged from -2.3% to 5.9% RE, and the intra-day precision ranged from 0.0 % to 1.3% RSD among the three concentrations. The inter-day accuracy ranged from -0.8% to 4.5% RE. The inter-day precision ranged from 1.5% to 2.0% RSD.

Ruggedness:

Ruggedness was conducted in a single validation run (n=6 at each concentration) using a different preparation analyst, a different instrument and on a different day. The intra-day accuracy ranged from 6.3% to 7.2% RE, and the intra-day precision ranged from 3.3 % to 3.9% RSD for matrix QC low, mid, and high samples.

Dilution:

As the highest potassium concentration in urine samples was anticipated to be below 4,000 µg/mL, the recovery for diluted samples was validated at 4,000 µg/mL (n=6) to accommodate analysing samples originally above the upper limit of the calibration range. Samples were diluted five fold with diluent to achieve a concentration within the calibration range. The average RE was 4.9%, and the RSD was 1.2%. Thus, both the accuracy and precision were demonstrated (RE ±15.0%, RSD ≤15.0%).

Matrix Effects:

Six different lots of human urine were fortified at 200 µg/mL and analysed. The responses of these samples, after the correction of endogenous contents, were compared to the response of fortified diluent solutions. The matrix factors (MF) ranged from 0.81 to 0.99 with an RSD of 7.8%. The matrix effect criterion was based on the precision (≤ 15%). The deviation of the analyte MF value from the unity (1.0) was linearly related to the urine lot with a higher degree of endogenous analyte, indicating such a deviation in MF values was from instrument response to high endogenous concentration, instead of from the matrix effect.

Stability Results:

The stability for potassium in human urine was evaluated for freeze/thaw, bench top storage, reinjection reproducibility (i.e., autosampler stability), and frozen storage, using QC high, mid (for autosampler stability only), low and the control with an acceptance of RE ±15.0%, RSD ≤15.0%. Stock solutions and calibration standard solutions were also evaluated for their ambient storage stability with acceptance criteria of relative difference (RD) ±10.0%, RSD ≤15.0%. Table 4 summarises the stability results in this validation study.

Conclusion

An ICP-MS assay for potassium in human urine was successfully validated by MPI Research. The method is accurate and precise for the determination of potassium in human urine over a calibration range of 100 to 2,000 µg/mL. The method was selective for the quantitation of potassium in human urine. Carryover does not impact the assay. Samples with a concentration up to 4,000 µg/mL can be accurately quantitated after dilution. Matrix samples are stable for at least eight freeze/thaw cycles, up to 25 hours at ambient temperature, up to 72 hours at ambient temperature in injection solution, and up to 243 days at 20°C.

The method has been successfully applied to the determination of potassium in human urine for several BE studies. This method is well suited

Table 4 Summary of the Validation Stability Results

Solution Type	Condition	Duration	Results
Calibration Stock Solution	Ambient	90 days	1.1%RD, 1.0%RSD
Quality Control Stock Solution	Ambient	91 days	0.2%RD, 1.0%RSD
Calibration Standards	Ambient	5 days	-4.6 to 1.2%RD; ≤2.7%RSD
Pre-processed Samples	Frozen (-20°C)	243 days	-5.6 to -1.2%RE, ≤1.2%RSD
	Ambient	25 hours	-2.7 to: -2.3%RE, ≤0.8%RSD
	Freeze/Thaw Cycles	8 cycles	-5.1 to -3.9%RE, ≤0.6%RSD
Post-processed Samples	Ambient	72 hours	-6.8 to -3.4%RE; ≤1.9%RSD

for routine clinical urine testing, as it covers the typical range of potassium in human urine. A quick turnaround and high throughput are expected in this method due to the wide linear dynamic range feature of ICP-MS. Indeed, the high efficiency in the ionisation process and the multi-elemental capability of ICP-MS renders this method to be less prone to matrix effects and drifts that commonly occur in other atomic spectroscopic techniques.

References

1. *www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM270390.pdf*
2. *Grunbaum, B.W. and Pace, N. Microchemical Urinalysis: VI. Determination of sodium, potassium, calcium, and magnesium by atomic absorption. Spectrophotometry in the microliter range of urine. Microchemical Journal, 1970, 15 :666-672*
3. *Khalil, S.A.H, Moody, G.J. and Thomas, J.D.R. Ion-Selective Electrode Determination of Sodium and Potassium in Blood and Urine. Analytical Letters, 1986, 19: 17-18*
4. *Krejsová, A., Černohorský, T. and Šurdová, E. Determination of sodium, potassium, magnesium and calcium in urine by inductively coupled plasma atomic emission spectrometry. The study of matrix effects. J. Anal. At. Spectrom., 2001, 16: 1002-1005*
5. *Food and Drug Administration (2001) Guidance for Industry: Bioanalytical Method Validation. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research*
6. *Food and Drug Administration (1978) 21CFR58 - Good Laboratory Practice for Nonclinical Laboratory Studies. Rockville, Maryland: US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=58*
7. *Shah, V.P., Midha, K.K., Findlay, J.W., Hill, H.M., Hulse, J.D., et al. (2000) Bioanalytical method validation - a revisit with a decade of progress. Pharmaceutical Research 17: 1551-1557*
8. *Viswanathan, C.T., Bansal, S., Booth, B., DeStefano, A.J., Rose, M.J., et al. (2007) Workshop/Conference Report - Quantitative Bioanalytical Methods Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays. The American Association of Pharmaceutical Scientists Journal 9: E30-E42*
9. *Shah, V.P. (2007) The History of Bioanalytical Method Validation and Regulation: Evolution of a Guidance Document on Bioanalytical Methods Validation. The American Association of Pharmaceutical Scientists Journal 9: E43-E47*
10. *Shah, V.P., Midha, K.K., Dighe, S., McGilveray, I.J., Skelly, J.P., et al. (1991) Analytical method validation: bioavailability, bioequivalence and pharmacokinetic studies. Conference report. European Journal of Drug Metabolism and Pharmacokinetics. 16: 249-255*GuidanceComplianceRegulatoryInformation/Guidances/UCM270390.pdf

Jennifer Ammerman, BS, Analytical Study Director/Manager at MPI Research, has nearly 10 years' experience serving in the CRO industry. She received her BS in chemistry from Juniata College in Huntingdon, Pennsylvania. In her current role, she oversees management of the laboratory and study direction for bioanalytical studies in the ICP-MS and GC-MS analytical platforms. Jennifer has authored multiple publications related to ICP-MS and bioanalysis.
Email info@mpiresearch.com

Chaoyang Huang, PhD, is a Senior Analytical Scientist at MPI Research. In this role, Dr. Huang is responsible for method development and validation of inductively coupled plasma mass spectrometry based analytical methods supporting drug discovery, formulation, clinical and nonclinical research and other related studies. Dr. Huang received his PhD in chemistry from Queen's University in Kingston, Canada, prior to joining MPI Research in 2006. He provides scientific expertise and solutions to analytical problems to meet our Sponsors' various needs and is published in various peer-reviewed journals.
Email info@mpiresearch.com

Daniel J. Wright, BS, joined MPI Research in 2007 and is the Director of Analytical Sciences at the MPI Research State College, PA facility. Mr. Wright has over 24 years of experience managing the operational and business aspects of contract analytical laboratories. He started as a bench chemist performing sample analysis by inductively coupled plasma atomic emission spectroscopy. He provides leadership and direction to the laboratory with an emphasis on laboratory automation, process improvement, and business development. Mr. Wright received his BS in biology from Manchester College in North Manchester, Indiana.
Email info@mpiresearch.com