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Full Validation of a High Resolution ICP-MS Bioanalysis Method for Iron in Human Plasma with K2EDTA

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Abstract

Purpose: To validate a bioanalytical inductively coupled plasma mass spectrometry (ICP-MS) method for quantification of iron in human plasma with K_2EDTA (matrix). This method was validated using typical bioanalytical validation parameters.

Materials and methods: Iron reference standards and germanium internal standard (IS) were from Ultra Scientific. Reagent water had a resistivity ≥18 MΩcm. Nitric acid and hydrochloric acid were ultra pure grade. Blank matrix was purchased from BioReclamation. Linearity was assessed using eight standards prepared in proxy matrix over a range equivalent to 0.5-20 µg iron/mL of matrix at the beginning and end of each run. Quality control (QC) samples were prepared by fortifying matrix at 0.5, 1.5, 10, 15 and 100 µg/mL. A blank containing IS and a double blank were included in every run. Selectivity was evaluated using six different lots of matrix fortified with IS and analyte at the LLOQ level. A Thermo-Finnigan ELEMENT2 High Resolution ICP-MS was used to analyze prepared samples.

Results: System suitability, selectivity, carryover, and ruggedness all met the acceptance criteria. Linearity was consistently demonstrated. Recoveries for all calibration standards and QCs were within the acceptance criteria. Freeze/thaw was demonstrated for 4 cycles. Bench top reproducibility was successful following 53 hours of ambient storage. Reinjection reproducibility was successful following 96 hours of ambient storage. Extract stability was successful following 190 hours of ambient storage. IS solution stability was successful following 29 days of ambient storage. Calibration standard stability was successful following 8 days of ambient storage. Analyte stock solution stability was successful following 29 days of ambient storage. Long term storage stability was successful following 31 days at 2 to 8°C, 31 days at -15 to -25°C, and 30 days at -70 to -90°C storage.

Conclusion: This bioanalytical ICP-MS method was successfully validated for the quantification of iron in human plasma with K_2EDTA.

Keywords: Inductively coupled plasma mass spectrometry; ICP-MS; Good laboratory practice; Nonclinical; Preclinical; Clinical; Bioanalytical method; Method validation; Iron; Human plasma

Abbreviations: Ar: Argon; As: Arsenic; Cd: Cadmium; CFR: Code of Federal Regulations; Cr: Chromium; Cu: Copper; EMEA: European Medicines Agency; EP: European Pharmacopoeia; EPA: Environmental Protection Agency; Diluent: Reagent water:HNO_3:HCl (490:5:5,v/v/v); FDA: Food and Drug Administration (United States); Fe: Iron; Ge: Germanium; GLP: Good Laboratory Practices; HCl: Hydrochloric acid; HNO_3: Nitric acid; Hg: Mercury; HR: High Resolution; ICP: Inductively coupled plasma; ICP-MS: Inductively coupled plasma mass spectrometry; Ir: Iridium; IS =InternalStandard; ISWS: Internal Standard Working Solution; LLOQ: Lower Limit of Quantitation; Matrix: Human plasma with K_2EDTA; Mn: Manganese; Mo: Molybdenum; Ni: Nickle; Os: Osmium; Pb: Lead; Pd: Palladium; Pt: Platinum; NIST: National Institute of Standards & Technology; QC: Quality Control; R^2: Coefficient of Determination; RD: Relative Deviation; RE: Relative Error; RF: Radio frequency; Rh: Rhodium; Ru: Ruthenium; RSD: Relative Standard Deviation; SOP: Standard Operating Procedure; SRM: Standard Reference Material; SST: System Suitability Test; ULOQ: Upper Limit of Quantitation; USP: United States Pharmacopeia; V: Vanadium

Introduction

Some heavy metals represent a serious health risk and are eliminated very slowly from the body. Heavy metal analysis has been performed under the auspices of the Environmental Protection Agency (EPA) [1,2] and the United States Pharmacopeia (USP) [3] for a long time. Early analytical techniques were based primarily on qualitative, non-specific, low-sensitivity colorimetric procedures based on the precipitation of insoluble metal sulfides. More recently, inductively coupled plasma mass spectrometry (ICP-MS) has been used to provide accurate, sensitive, specific quantification of heavy metals in environmental samples, forensic samples, nutraceutical products, and drug articles (drug substances, drug products, and excipients)[4-9]. The main advantages of ICP-MS over the classical methods are its ability to accurately quantify trace metals at much lower detection limits, analytical speed, relative lack of interference, and definitive multiple isotope capability. The USP has identified four heavy metal contaminants known or suspected to pose a significant

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safety concern (human carcinogens) as Class 1 elements that must be quantified in dietary supplements and their ingredients, as well as in drug substances and excipients based on their toxicologically relevant limits: arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg). Metals of low safety concern are Class 2 impurities that are generally well tolerated at typical pharmaceutical exposures and include catalysts which may have been introduced during processing, such as chromium (Cr), copper (Cu), manganese (Mn), molybdenum (Mo), nickel (Ni), palladium (Pd), platinum (Pt), vanadium (V), osmium (Os), rhodium (Rh), ruthenium (Ru), and iridium (Ir). Metals of minimal safety concern are Class 3 impurities that have well established safety profiles, no significant toxicity, are well tolerated, and typically are ubiquitous in nature, such as iron (Fe). Limits for each element are based upon European Medicines Agency (EMEA) guidelines [10]. More recently, sample analysis of metals using ICP-MS has been performed in support of Good Laboratory Practice (GLP) nonclinical (preclinical) and clinical toxicology safety studies. In some cases, metals are an intrinsic component of the drug substance and therefore may be used to provide a means for accurate quantitation of the exposure for that particular material based on the elemental concentration in biological samples.

The primary objective of this study was to validate an analytical method for the quantitative determination of iron in human plasma with K_2EDTA (matrix) using high resolution (HR) ICP-MS technology. The method was designed for quantitation of a drug substance containing an iron core after administration to human patients, but it may be applicable to any analysis for iron in human plasma, no matter the origin. This method was validated in accordance with the United States Food and Drug Administration (FDA) Guidance for Industry for Bioanalytical Method Validation [11], 21 Code of Federal Regulations (CFR) Part 58 GLP regulations [12], the industry leading bioanalytical method validation meeting reports [13-16], and current MPI Research standard operating procedures (SOPs). The EMEA Guideline on bioanalytical method validation [17] was used as a reference. Since this study is intended for use in support of GLP clinical toxicology safety studies, the MPI Research Quality Assurance Unit was assigned to review all phases of the study, including, but not limited to, analyst GLP-compliant bioanalytical validation and ICP-MS training, instrument validation and calibration, protocol and amendments, routine laboratory inspections, and draft and final reports.

This method was validated for the purpose of quantitating iron content in human plasma samples obtained from clinical studies with patients dosed with an active pharmaceutical ingredient that contains iron. Previous studies have been performed that include iron in a multi-element ICP-MS assay in blood, serum or biological samples [18-22]. The assay described in this report was intended to bring bioanalytical acceptance criteria and study design to an ICP-MS analysis for the single element, a platform that is historically applied in the environmental analysis.

Calibration Standard Identification	Volumes Used (mL)		Total Volume (mL)	Final Concentration (µg/mL)
	100 or 1000 µg/mL Fe	Diluent		
S0	0	1.000	1.000	0
S1	0.005 (100)	0.995	1.000	0.5
S2	0.010 (100)	0.990	1.000	1
S3	0.020 (100)	0.980	1.000	2
S4	0.005 (1000)	0.995	1.000	5
S5	0.010 (1000)	0.990	1.000	10
S6	0.015 (1000)	0.985	1.000	15
S7	0.018 (1000)	0.982	1.000	18
S8	0.020 (1000)	0.980	1.000	20

Table 1: Calibration Standard Preparation.

QC Sample Identification	Volumes Used (mL)		Total Volume (mL)	Fortified Iron Concentration In Matrix (µg/mL)
	100, 1000 or 10,000 µg/mL Fe	Matrix		
QC Control (i.e., no Fe)	0.000	1.000	1.000	0
LLOQ	0.005 (100)	0.995	1.000	0.5
QC Low	0.015 (100)	0.985	1.000	1.5
QC Mid	0.010 (1000)	0.990	1.000	10
QC High	0.015 (1000)	0.985	1.000	15
QC Dilution	0.010 (10,000)	0.990	1.000	100

Table 2: QC Sample Preparation.

This bioanalytical ICP-MS method for quantification of iron in human plasma with K_2EDTA was validated over a range of 0.5 to 20 µg/mL. The method was evaluated for its capability to quantify iron in human plasma with K_2EDTA samples that contain iron up to 100 µg/mL, after dilution. Stability was evaluated after storage for one month in refrigerated (2 to 8°C), frozen (-15 to -25°C) and ultralow frozen (-70 to -90°C) conditions.

Materials and Methods

Analyte, Internal standard, Matrix, and reagent information

The Iron inductively coupled plasma (ICP) Standards, used as the analyte and reference standard (1000 µg/mL and 10,000 µg/mL, respectively), were ULTRAgrade™ Solutions purchased from Ultra Scientific, North Kingstown, Rhode Island. The Germanium ICP Standard, used as the internal standard (IS, 1000 µg/mL), was an ULTRAgrade™ Solution purchased from Ultra Scientific, North Kingstown, Rhode Island. The blank human plasma with K_2EDTA, lipemic human plasma with K_2EDTA, hemolyzed human plasma with K_2EDTA, and Type II diabetic human plasma with K_2EDTA were purchased from BioReclamation, Westbury, New York. The Type II diabetic plasma had concomitant medications of Synthroid, allopurinol, Lasix, amlodipine, metoprolol, vitamin D, Avodart, Zocor, and Levemir. A standard reference material (SRM, 1598a) for inorganic constituents in animal serum was derived from healthy bovine and porcine animals and obtained from the National Institute of Standards & Technology (NIST), Gaithersburg, Maryland. Ultrex, ultra-pure grade, nitric acid (HNO_3) and hydrochloric acid (HCl) were obtained from J.T. Baker, Mallinckrodt Baker, Inc., Phillipsburg, New Jersey. Reagent water with a resistivity ≥18 MΩcm was obtained using a Diamond™ RO reversed osmosis system from Barnstead International, Dubuque, Iowa.

Iron (Fe) m/z	57
Germanium (Ge) m/z	72
Radio frequency (RF) power	1.3 kW
Argon (Ar) cool gas flow	Typically 16 L/min
Ar plasma gas flow	Typically 0.80 L/min
Ar nebulizer gas flow	Typically 0.8 – 1.1 L/min
Nebulizer	PFA-100 micro flow
Spray Chamber	Cyclonic
Detector mode	Dual
Measurement units	cps
Peristaltic pump speed	12 R/min
Peristaltic Pump Tubing	Black-black (0.76 mm i.d. x 150 mm 2-stop, PVC)
Sample uptake time	120 s
Wash time	120 s
Resolution mode	Medium
Sample time	20 ms
Samples/peak	30
Mass Window	300 (^{57}Fe and ^{72}Ge)
Search Window	60 (^{57}Fe), 10 (^{72}Ge)
Integration Window	20(^{57}Fe), 20(^{72}Ge)
Acquisition points	10
Scan type	EScan

**The parameters were adjusted to optimize system response on a run-by-run basis.*

Table 3: Typical HR ICP-MS Instrument Settings.

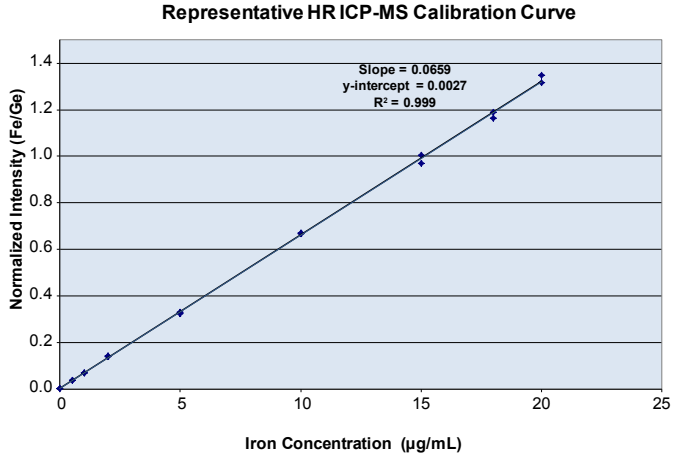

Figure 1: Representative HR ICP-MS Calibration Curve.

Method limitations

This method is for the determination of iron (^{57}Fe) in human plasma with K_2EDTA at concentrations ranging from 0.5 to 20 µg/mL. Samples with concentrations above the upper limit of quantitation (ULOQ) may be diluted within the range of the calibration curve, processed, and then analyzed. Due to the endogenous concentrations of iron in human plasma, the calibration curve was prepared in a proxy matrix of reagent water:HNO_3:HCl (490:5:5,v/v/v) (diluent).

Control materials used for preparation of quality control (QC) samples were prescreened for endogenous concentrations of iron. A control material was suitable for use if the endogenous concentration was less than the lower limit of quantitation (LLOQ) of 0.5 µg/mL. If the control material did not meet this requirement, it was not used to prepare QC samples.

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

Centrifuge tube pre-treatment and pre-screening

All 15 mL centrifuge tubes were pre-treated and pre-screened before use. All tubes and caps were soaked for a minimum of 12 hours with a 1% HNO_3 in water wash solution in a covered container. The tubes and caps were rinsed with reagent water with a resistivity ≥18 MΩcm. Tubes and caps were racked and allowed to air dry for pre-screening. Approximately 10 mL of diluent was added to each pre-treated tube, and tubes were capped and mixed on a Roto-shake Genie at the maximum speed for 2 minutes. The diluent from each tube was then analyzed by HR ICP-MS, along with the first standard curve sample. The acceptance criteria was ≤20% of the total iron response in the first standard curve sample. Tubes that failed this limit were discarded. Tubes that met this limit were rinsed with reagent water, racked, and allowed to air dry.

Preparation of solutions and samples

Internal Standard Working Solution (ISWS): A 100 µL aliquot of purchased 1000 µg/mL germanium was added to a 50 mL polypropylene DigiTube and approximately 20 mL water and 0.5 mL nitric acid were added to the tube. The final volume was brought up to 50 mL with water, and the solution was mixed thoroughly by inversion.

Calibration and QC stock solutions: Two equivalent solutions were prepared: one used to prepare the calibration curve and one

used to prepare the QC samples. A 500 µL aliquot of purchased 10,000 µg/mL iron primary standard solution was added to a 50 mL polypropylene DigiTube and then approximately 20 mL reagent water and 1.0 mL nitric acid were each added to the tube. The final volume was brought up to 50 mL with reagent water, and then the solution was mixed thoroughly by inversion.

Calibration standards: Calibration standards ranging from 0.5 µg/mL to 20 µg/mL iron were prepared in reagent water:HNO_3:HCl (490:5:5,v/v/v) according to the following table. Each prepared solution was mixed thoroughly by vortexing. A single set of calibration standards was prepared and then analyzed in duplicate as one set at the beginning and one set at the end of the sample batch.

QC samples: QC samples at the LLOQ, low, mid, high, and dilution QC concentrations were prepared in matrix according to the following table. All samples were mixed thoroughly by vortexing. Dilution QC samples were diluted 10-fold with diluent prior to the sample preparation and analysis.

Extraction procedure

1. Matrix was thawed unassisted at ambient temperature.
2. Matrix samples were mixed by vortexing.
3. 100 µL aliquots of QC control, QC sample, or calibration standard were added to pre-screened 15.0 mL centrifuge tubes.
4. 100 µL aliquots of ISWS were added to all tubes except the double blank tubes.
5. 100 µL aliquots of diluent were added to the double blank tubes.
6. 9.80 mL aliquots of diluent were added to each tube. Tubes were capped and the contents mixed thoroughly by inversion on the Roto-shake Genie at the maximum speed for 2 minutes.
7. Samples were analyzed by HR ICP-MS.

Equipment and HR ICP-MS instrument settings

A Thermo-Finnigan ELEMENT2 HR ICP-MS equipped with an autosampler and Thermo ELEMENT2 software version 2.41 data acquisition system was used:

Analytical run acceptance criteria

System suitability test (SST) solution: An additional calibration standard at the LLOQ concentration was prepared and used as the SST solution. The analyte response (^{57}Fe) in the SST solution was at least 10 times the response in the diluent. The instrument was operated at a medium resolution mode. The mass resolution at this mode was at least 2500 for ^{57}Fe. The third component of the SST evaluation was the analysis of a calibration curve. The SST calibration curve was calculated as the response ratio (analyte response/IS response) versus the concentration of analyte using least squares analysis. The acceptance criteria for linear regression analysis were 1) all SST calibration standards within ±10.0% relative error (RE) of the nominal concentration (±15.0% at LLOQ), 2) coefficient of determination (R^2) ≥0.990, and 3) all SST calibration standards must be used in the regression analysis.

Calibration curve: The calibration curve for each run was calculated as the response ratio (analyte response/IS response) versus the concentration of analyte using least squares analysis. The acceptance criteria for linear regression analysis, including the calculated concentrations of the calibration standards, were 1) all calibration standards within ±10.0% RE of the nominal concentration (±15.0% at LLOQ), 2) R^2 ≥0.990, and 3) all calibration standards must be used in the regression analysis.

Reagent double blank: A reagent double blank (the diluent with no IS) was included in each analytical run for the evaluation of possible carryover. The reagent double blanks should be located after the ULOQ and should not exceed 20.0% of the mean analyte response of the LLOQ standards and 5.0% of the mean internal standard response in the LLOQ standards.

System performance: The system performance was monitored throughout the analytical run by periodically injecting the QC samples. The results of the QC samples provide the basis of accepting or rejecting the run. At least 66.7% of all QC samples and 50% at each level should be within ±15.0% RE of their respective nominal concentration.

Results and Discussion

System suitability test

Prior to each validation run, an SST solution (LLOQ calibration standard) was injected (n=1) and compared to the response of a diluent injection. The analyte (^{57}Fe) response of each SST injection was at least 10 times the response of the diluent injection in each run. Additionally, the mass resolution in medium resolution mode was at least 2500 for ^{57}Fe. The third component of the SST evaluation was the analysis of a calibration curve. All SST calibration standards were used in the regression analysis, with a R^2 ≥0.990, and all SST calibration standards were within ±10.0% RE of the nominal (±15.0% RE at LLOQ). All SST criteria were met for all reported runs.

Selectivity

Eight lots of matrix were evaluated for selectivity. Among these lots, the following special considerations were included: at least one lot of lipemic plasma, at least one lot of hemolytic plasma, at least one plasma lot from a male over age 60 with Type II Diabetes, and at least three lots of plasma from males and three from females. None of the eight lots of matrix tested for selectivity had a significant response for the IS (IS peak response in plasma blanks ≤5.0% of the mean IS response in the LLOQ standards). Upon final testing, all analyzed matrix types successfully met the acceptance criteria for mean accuracy within ±20.0% RE of the nominal concentration when fortified at the LLOQ concentration. Therefore, the method was selective for iron and the IS. However, precaution should be taken with sample collection to not lyse the red blood cells, thus releasing more iron into the plasma and elevating the total iron content above typical endogenous levels.

Linearity

Due to the endogenous concentration of iron in human plasma, calibration curves were prepared in a proxy matrix of 5:5:490 HNO_3:HCl:reagent water (v:v:v). Two calibration curves were injected: one at the beginning and one at the end of each validation run. A single regression was generated from the two calibration curves. All calibration curves met the validation acceptance criteria (100% of all points used in regression, a minimum of eight standard levels, and curve points within ±10.0% RE, 15.0% RE at LLOQ).

Linear regression with no weighting was used for this validation. The calibration curves had consistent slope with little variability observed in the intercept and R^2 of ≥0.999, which met the acceptance criterion (R^2 ≥0.990).

Carryover

Carryover was assessed by injection of two reagent double blanks after each ULOQ calibration standard for all but two accepted validation runs. The acceptance criteria for the carryover blanks were a response for iron ≤20.0% compared to the mean iron response in the acceptable LLOQ standards and a response for the IS ≤5.0% compared to the mean IS response in the acceptable LLOQ standards. Carryover does not impact this assay.

Evaluation of standard reference material

The SRM purchased from NIST were analyzed (n=6) in each main validation run. The SRM was prepared using healthy bovine and porcine serum fortified with various inorganic constituents, including the certified iron concentration of 1680 ±60 µg/L. Although the SRM was not the same matrix as the remainder of the validation samples, the SRM was used to show the ability of the proxy matrix calibration curve to accurately quantitate iron in a biological matrix.

The acceptance criteria were a mean concentration within ≤10.0% RE of the nominal and ≤10.0% relative standard deviation (RSD). The SRM was analyzed in three data sets (total n = 18). The average % RE ranged from -1.1% to 3.6%, and the RSD was ≤5.4%. The results show that the iron content in the SRM can be accurately quantified using the proxy matrix calibration curve.

Accuracy and precision

The intra-day accuracy of the QC samples (n=6) was determined for each validation run. The inter-day accuracy of the QC samples was determined across the three core validation runs and the ruggedness run. The acceptance criterion was a mean concentration within ±15.0% RE of the nominal concentration for each level (±20.0% for LLOQ). For the LLOQ samples (0.5 µg/mL), the intra-day accuracy for the three core validation runs ranged from -10.4% to 2.2%, and the inter-day accuracy was -1.4%. For the QC Low samples (1.5 µg/mL), the intra-day accuracy for the three core validation runs ranged from -5.0% to -1.9%, and the inter-day accuracy was -2.9%. For the QC Mid samples (10 µg/mL), the intra-day accuracy ranged from -2.6% to -0.4%, and the inter-day accuracy was -1.1%. For the QC High samples (15 µg/mL), the intra-day accuracy ranged from -2.3% to -0.6%, and the inter-day accuracy was -1.6%. All results met the acceptance criterion.

The intra-day precision of the QC samples was determined for each validation run that contained at least three replicates at each QC level. The inter-day precision of the QC samples was determined across the three core validation runs and the ruggedness run. The acceptance criterion was a RSD ≤15.0% for each level (≤20.0% for LLOQ). For the LLOQ samples, the intra-day precision ranged from 2.0% to 10.0%, and the inter-day precision was 9.1%. For the QC Low samples, the intra-day precision ranged from 2.4% to 2.7%, and the inter-day precision was 3.5%. For the QC Mid samples, the intra-day precision ranged from 2.0% to 4.0%, and the inter-day precision was 2.7%. For the QC High samples, the intra-day precision ranged from 2.4% to 3.6%, and the inter-day precision was 3.0%. All results met the acceptance criterion.

Additional runs beyond the core validation runs and the ruggedness run contained at least duplicate QC samples at each concentration level. These QC samples were used solely to accept or reject the runs. The runs were considered valid if at least 66.7% of all QC samples and at least 50% of the QC samples at each level were within ±15.0% RE of the nominal value.

Dilution

The recovery for diluted samples was validated at 100 µg/mL (n=6) to accommodate analyzing samples originally above the upper limit of the calibration range. Samples were diluted 10-fold with diluent to achieve a concentration within the calibration range and were analyzed on the day of preparation. The average RE was 2.2%, and the RSD was 1.3%. Since the accuracy and precision were both demonstrated (RE ±15.0%, RSD ≤15.0%), up to a 10-fold dilution of the sample does not compromise quantitation of the iron in human plasma.

Ruggedness

Ruggedness was analyzed in one data set (n=6 at each concentration) using a different preparation analyst from the three core validation runs. The acceptance criteria were a mean concentration within ±15.0% RE of the nominal (±20.0% for LLOQ) and ≤15.0% RSD (≤20.0% for LLOQ). The average RE for the LLOQ samples was 5.2%, and the RSD was 5.2%. The average RE for the QC Low samples was -0.7%, and the RSD was 4.6%. The average RE for the QC Mid samples was -0.8%, and the RSD was 2.2%. The average RE for the QC High samples was -1.9%, and the RSD was 3.6%. All acceptance criteria were met; therefore, the ruggedness of the assay was demonstrated.

Stability results

Freeze/Thaw Cycle: The QC samples at low and high concentrations were evaluated (n=6) after four freeze/thaw cycles, and the mean results were compared to the nominal values. The average RE for the QC Low samples was -6.9%, and the RSD was 5.0%. The average RE for the QC High samples was -0.4%, and the RSD was 3.8%. The samples were shown to be stable over four freeze/thaw cycles because the average RE at each level was within ±15.0% of the respective nominal values, and the RSD at each level was ≤15.0%.

Bench Top Stability: Bench top stability for QC samples at low and high concentrations was evaluated (n=6) after 53 hours of ambient storage. The mean results were compared to the nominal concentrations. The average RE for the QC Low samples was -3.3%, and the RSD was 3.7%. The average RE for the QC High samples was -0.7%, and the RSD was 3.8%. The average RE at each level was within ±15.0% of the respective nominal values, and the RSD at each level did not exceed 15.0%. Human plasma samples containing iron are stable for up to 53 hours at ambient temperature.

Reinjection Reproducibility: Reinjection reproducibility (post-processed stability) was evaluated by re-injection of QC samples (n=6) and calibration standards that were stored for 96 hours at ambient conditions. The calibration standards from the original injection were used to quantitate the reinjected QC samples. The reinjection reproducibility QC samples met the validation acceptance criteria for accuracy (average calculated concentration within ±15.0% RE of the nominal concentration) and precision (calculated concentration ≤15.0% RSD). Samples can be maintained at ambient conditions for up to 96 hours in injection solution without adversely affecting the accurate quantitation of iron using aged calibration standards. The average RE for the QC Low samples was -7.2% with a RSD of 3.4%. The average RE for the QC Mid samples was -2.4% with a RSD of 2.3%. The average RE for the QC High samples was -1.1% with a RSD of 2.9%.

Extract Stability: Extract stability was evaluated by analyzing aged extracted QC samples at the Low and High concentrations (n=6) that were stored for 190 hours at ambient conditions. QC samples were quantitated using freshly prepared calibration standards. The extract

stability QC samples met the validation acceptance criteria for accuracy (average calculated concentration within ±15.0% RE of the nominal concentration) and precision (calculated concentration ≤15.0% RSD). Samples can be maintained at ambient conditions for up to 190 hours in injection solution without adversely affecting the accurate quantitation of iron using freshly prepared calibration standards. The average RE for the QC Low samples was -5.2% with a RSD of 3.2%. The average RE for the QC High samples was 0.2% with a RSD of 2.2%.

Stock Solution Stability: Ambient iron stock solution and ISWS stability was evaluated at 29 days by comparing a fresh solution to the aged solution (n=6). The ambient solutions met the acceptance criteria (within ±7.0% relative deviation [RD] for iron and within ±20.0% RD for IS, ≤15.0% RSD). Iron stock and ISWS solutions are stable for up to 29 days at ambient temperature. The % RD for the analyte was -1.4% with RSDs ≤4.7%. The % RD for the ISWS was -1.1% with RSDs ≤1.0%.

Ambient stability of the lowest and highest calibration standard solutions was evaluated at 8 days by comparing a fresh solution to the aged solution (n=6). The ambient solutions met the acceptance criteria (within ±7.0% RD, ≤15.0% RSD). Iron is stable in the calibration solutions for up to 8 days at ambient temperature. The % RD was -0.4% and 3.1% for the low and high calibration solutions, respectively. The RSDs were ≤6.8% for all solutions analyzed.

Long Term Storage Stability: Long term storage stability QC samples (n=6) were stored for intervals of approximately 1 month (31 days) at 2 to 8°C and at -15 to -25°C (hereafter referred to as 4°C and -20°C). QC samples were also stored for 30 days at -70 to -90°C (hereafter referred to as -80°C). The samples were evaluated against a fresh calibration curve, and the average values were compared with the nominal concentration.

At 4°C, the RE for QC Low samples was 0.6%, and the RSD was 3.7%. The RE for the QC High samples was 4.6%, and the RSD was 2.7%. At -20°C, the RE for QC Low samples was 2.9%, and the RSD was 2.8%. The RE for the QC High samples was 3.9%, and the RSD was 3.9%. At -80°C, the RE for QC Low samples was -8.4%, and the RSD was 2.5%. The RE for the QC High samples was -5.1%, and the RSD was 1.2%.

The samples were shown to be stable for at least 31 days when stored at both 4°C and -20°C and for at least 30 days at -80°C at each concentration assessed, because the average RE for each level was within ±15.0% and the RSD did not exceed 15.0%.

Conclusion

An HR ICP-MS assay for iron in human plasma with K_2EDTA was successfully validated by MPI Research. The method is accurate and precise for the determination of iron in human plasma with K_2EDTA over the validated calibration range of 0.5 to 20 µg/mL. The method was selective for the quantitation of iron and the IS in human plasma with K_2EDTA. The ability to quantify iron in the presence of specialized plasma sources is not adversely affected based on the evaluation of this method. Carryover does not affect the accurate quantitation of iron. Samples with a concentration above the ULOQ can be accurately quantitated by diluting the samples up to a 10-fold dilution with diluent to achieve a concentration within the range of the calibration curve. Samples can be exposed for up to four freeze/thaw cycles in human plasma with K_2EDTA without adversely affecting the accurate quantitation of iron. Samples can be exposed for up to 53 hours at ambient temperature in human plasma with K_2EDTA without adversely affecting the accurate quantitation of iron. Samples can be exposed for up to 96 hours stored at ambient temperature in injection solution without adversely affecting the accurate quantitation of iron using aged calibration standards. Samples can be exposed for up to 190 hours stored at ambient temperature in injection solution without adversely affecting the accurate quantitation of iron using freshly prepared calibration standards. Iron stock and calibration solutions were stable for up to 29 and 8 days, respectively, at ambient temperature. ISWS was stable for up to 29 days stored at ambient temperature. Samples can be stored for up to 31, 31, or 30 days at 4°C, -20°C, or -80°C, respectively, in human plasma with K_2EDTA without adversely affecting the accurate quantitation of iron.

We successfully validated an HR ICP-MS bioanalytical method for the quantification of iron in human plasma with K_2EDTA matrix by employing critical aspects of GLPs, bioanalytical guidances, and compendia criteria.

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