Development and Validation of an Analytical Method for Total Thallium in Rodent Plasma and Tissues by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

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Abstract

Thallium (Tl) is a naturally occurring element that is used in the semiconductor industry and manufacturing of optics and electronics, resulting in potential human exposure. Because there are knowledge gaps for the toxicity of Tl compounds, the National Toxicology Program (NTP) is investigating the toxicity of Tl_2SO_4 in rodents. The objective of this work was to develop and validate a method to quantitate total Tl in rodent plasma and other matrices in support of NTP studies.

Male Sprague Dawley (SD) rat Plasma was digested with nitric acid and hydrogen peroxide, and analyzed by inductively-coupled plasma mass spectrometry (ICP-MS). The method was successfully validated over the concentration range of 1.25 to 500 ng Tl/mL. Linearity and curve fit were demonstrated with correlation coefficient (r) ≥ 0.99 and percent relative error (%RE) ≤ ±5% for all matrix standards. The limit of detection (LOD) was established as 0.0370 ng/mL. Acceptable intra- and inter-day precision and accuracy were demonstrated (%RSD ≤ 4.3%, mean %RE ≤ ±5.6%). The method was evaluated in several secondary matrices including Postnatal Day 4 (PND4) Harlan Sprague Dawley (HSD) dam and pup plasma, gestational day (GD) 18 HSD rat fetal homogenate, HSD rat urine, female HSD rat brain homogenate, and female B6C3F1/N mouse plasma. Background Tl was detected in fetal and brain homogenates and urine, but the response was <30% of the response for the lower limit of quantitation (LLOQ) and did not interfere with method performance. These results demonstrate that the method is suitable for the quantitation of Tl in rodent matrices generated from toxicology studies.

Background

Thallium – Exposure Factors and Toxicology

- Used in manufacture of industrial products (optics, electronics, etc.) and was historically used as a rat poison (outlawed in the US in 1972).[1]
- Byproduct of metal refining activities (e.g., iron, cadmium, zinc).
- Highly toxic ($TlSO_4$ oral LD_{50} = ~25 mg/kg body weight in mice), but reference doses have not been established.[2]
- Toxicology studies needed to guide regulatory decisions; these studies rely on validated analytical methods for collection of scientifically-defensible data.[3]

Research Objectives

- Develop and validate bioanalytical method for determination of total Tl concentration in male SD rat plasma.
- Development: Total Tl in digested rat plasma was analyzed in several matrices to investigate potential matrix effects on quantification and optimize digestion parameters.
- Validation: Bioanalytical method was validated, and linearity, accuracy, precision, recovery, selectivity, analytical limits, and dilution verification were established.
- Analyte stability was also assessed under several scenarios: analysis period stability in plasma extract (refrigerator and autosampler) and plasma matrix (freeze-thaw and frozen-storage), and in frozen secondary matrices (PND4 dam and pup plasma, GD 18 fetus, rat urine, female rat brain, and female mouse plasma).

Materials and Methods

Instrumentation

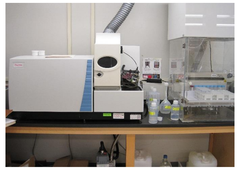
Sample digestion: DigiPREP heated graphite block (SCP Science, NY)

Sample analysis: X-Series II (Thermo) w/ Peltier-cooled glass impact bead spray chamber

Materials

Primary matrix: Control Sprague Dawley rat plasma (Bioreclamation IVT, Westbury, NY; male, 8-12 weeks, pooled from 100 animals), stored at ~-80 °C until use

Secondary matrices: Control PND4 Harlan Sprague Dawley (HSD) dam and pup plasma GD 18 HSD rat fetus (homogenized for analysis), HSD rat urine, female HSD rat brain (homogenized for analysis), and female B6C3F1/N mouse plasma (BioIVT, Westbury, NY)



1,000 and 10 µg/mL NIST-traceable standards of Tl (High Purity Standards, Charleston, SC) and 1,000 µg/mL Pr standard (High Purity Standards) for use as an internal standard

Sample preparation: 30% hydrogen peroxide (H_2O_2, SupraPur purity, non-stabilized, EMD Millipore, Burlington, MA, USA), concentrated nitric acid (HNO_3, Trace Metal Grade purity, Fisher, Hampton, NH, USA), and deionized water (~18 MΩ cm-1)

Sample Preparation

Digestion mixture: 10:1 ratio of Trace Metal Grade purity HNO_3 (1.00 mL for samples):plasma (0.100 mL for samples) mixed in vials

Standards and QC samples spiked with Tl intermediate stock standard before digestion

Materials and Methods (continued)

Digestion: Digitally-controlled, graphite heating block at 95 °C for 30 minutes. Allow to cool to ambient temperature, add H_2O_2 at 5 equivalents plasma volume (0.500 mL), cap and digest at 95 °C for 30 minutes. Allow to cool to ambient temperature, add internal standard stock solution (5 ng/mL final nominal concentration), dilute to volume w/ deionized water (5 mL) and mix.

ICP-MS Analysis - Validation

Method development experiments established that plasma matrix calibration was preferable to digestion solvent matrix for quantitation of Tl in biomatrices.

Standards and digested samples were analyzed for total Tl by ICP-MS. Typical system conditions during validation are shown below. Parameters were optimized daily with a tuning solution.

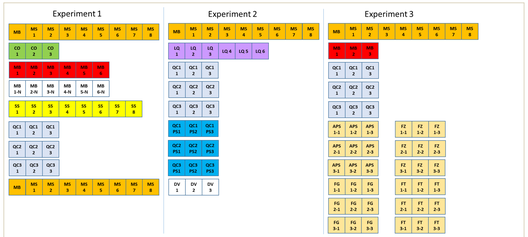
Instrument and Conditions

Instrument:	Thermo X-Series II ICP-MS (Waltham, MA)
Software:	Thermo Plasma Lab ICP-MS Software
Isotopes Monitored:	^{205}Tl (analytical), ^{141}Pr (internal standard)
Injector:	1.8 mm, quartz
Spray Chamber:	Glass impact bead
Cones:	Xt

Method Validation

A calibration curve was prepared in plasma over the range 1.25-500 ng/mL on each of 3 separate days (defined as core validation Experiments 1, 2, and 3). Parameters assessed: linearity, precision, accuracy, recovery, selectivity, analytical limits, and analysis period stability. Additionally, dilution integrity and short-term and long-term storage stability, up to ~30 and ~60 days, respectively, were evaluated.

Scheme 1. Diagram demonstrating the proposed core validation parameters for thallium



MS 1	Matrix standard. Can be solvent standard on days 2 and 3 if matrix impact shown to be insignificant.
CO 1	Carryover blank; same as matrix blank
MB 1	Matrix blank; internal standard.
MB 1-N	Matrix blank; no internal standard.
SS 1	Solvent standard. Prepared at same nominal concentrations as matrix standards.
QC1 1	Matrix quality control sample.
LLQ 1	LLOQ evaluation.
QC1 PS1	Matrix standard; matrix impact. These are fortified at same levels as matrix standards, above, but after digestion.
DV 1	Dilution verification sample; prepared outside of calibration range and diluted to mid-range with digested control matrix.
APS L1	Analysis period stability sample. APS: autosampler ambient storage reanalysis; FG: refrigerator extract storage stability; FZ: freezer sample storage stability; FT: freeze-thaw cycle sample stability.

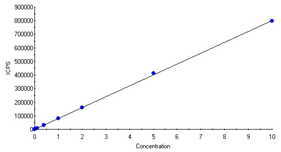
Method Validation Results

- Limits/sensitivity: LLOQ was lowest concentration standard with acceptable %RE and %RSD for 6 replicates; Limit of detection was calculated as 3 x SD of 6 replicate samples fortified at LLOQ
- LLOQ = 1.25 ng Tl/mL plasma; LOD = 0.037 ng Tl/mL plasma
- Matrix blank analysis: Average 0.358 ng Tl/mL plasma background measured

Results

Method Validation Results (continued)

- **Linearity**



Parameter	Experiment Day	Matrix Calibration Values
Linear Range (ng Tl/mL plasma)	1 2 3	1.25 – 500 1.25 - 500 1.25 - 500
Slope	1 2 3	79935 78370 87141
y-Intercept	1 2 3	724 -93.2 -227
Correlation Coefficient (r)	1 2 3	0.9999 0.9999 0.9999

- Acceptable linearity was demonstrated for matrix calibration on all validation days.

- **Extraction Recovery and Matrix Impact**
- Extraction Recovery: Matrix and solvent calibration curves analyzed, extraction recovery calculated across calibration range: 97.8-104%, 101% average, range of 6%.
- Pre-digestion and post-digestion Tl-spiked plasma samples were compared, average matrix impact: -0.5%.

- **Method Selectivity**
- Selectivity: comparison of Tl background signal in matrix blanks to measured Tl signal in LLOQ standards.
- Average selectivity for n=6 MB samples and n=6 LLOQ standards: 6%.

- **Instrument Drift**
- Instrument drift was measured by analyzing the matrix calibration curve both at the beginning and the end of the Day 1 analysis sequence.
- Comparison of measured standard concentrations at beginning and end: Range: -5.3 - -1.4% drift at all levels.

- **Accuracy and Precision**
- Acceptable interday and intraday precision and accuracy were demonstrated (mean %RE ≥ ±6.4%; %RSD ≤ 5.4%) for matrix QC samples.

	Intraday Precision & Accuracy		Interday Precision & Accuracy	
Target [Tl] (ng Tl/mL plasma)	Intraday Accuracy Mean %RE	Intraday Precision %RSD	Interday Accuracy Mean %RE	Interday Precision %RSD
2.00	-3.5	0.8	-4.8	1.3
50.0	-5.6	0.2	-2.4	4.3
400	-1.7	0.6	-1.3	0.9
10,000[a]	-6.4	5.4	NA	NA

[a] Note that these samples were prepared outside of the linear range and were analyzed to monitor dilution effects on method accuracy. Samples were only prepared on one validation day (Day 2), so interday accuracy and precision were not assessed for this concentration level.

- **Storage Stability Study Results**

Storage Stability Results				
Stability type	**Nominal conc. (ng/mL)**	**Mean found conc. (ng/mL)**	**Mean % of (Reference)**	**%RSD**
Reference concentrations (Day 0 Results)	2.00	1.88	NA	0.9
	50.0	48.4	NA	0.6
	400	392	NA	0.2
Extract: Ambient 5 days	2.00	1.89	101%	2.6
	50.0	47.6	98.3%	0.3
	400	391	99.7%	0.1
Extract: Refrigerator 5 days	2.00	1.88	99.7%	0.7
	50.0	47.5	98.2%	0.3
	400	393	100%	0.3
Spiked Plasma: Freezer 5 days	2.00	1.86	98.9%	0.3
	50.0	47.7	98.5%	0.9
	400	389	99.2	0.6
Spiked Plasma: Freeze/Thaw 3 cycles over 3 days	2.00	1.90	101%	1.6
	50.0	47.2	97.5%	0.7
	400	389	99.0%	0.5

- **Secondary Matrix validation**
- Secondary Matrix Specificity (as Tl signal in unfortified control secondary matrix compared to LLOQ fortified primary matrix standard) ranged from 2.37 – 5.37% of LLOQ for rat and mouse plasma matrices.
- Background Tl was detected in urine, brain, and fetus matrix samples with signal intensities ranging from 16.9-24.3% of LLOQ signal.

Results (continued)

- **Secondary Matrix validation**
- Secondary matrices were fortified with Tl and analyzed against plasma matrix calibration curve, 6 replicates at 160% LLOQ.

Secondary Matrix Validation Results				
Secondary Matrix	**Nominal conc. (ng/mL)**	**Mean found conc. (ng/mL)**	**Mean % Relative Error**	**%RSD**
Adult Female HSD Rat Plasma (PND4)	2.00	1.98	-1.1%	0.5%
Female HSD Rat Pup Plasma (PND4)	2.00	1.99	-0.3%	0.9%
Female HSD Rat Urine	2.00	2.18	9.1%	0.7%
Female B6C3F1 Mouse Plasma	2.00	1.98	-1.2%	1.5%
Homogenized HSD Rat Fetus	2.00	2.18	9.2%	1.4%
Homogenized HSD Rat Brain	2.00	2.25	12.5%	1.5%

- **Secondary Matrix Storage stability (60-day)**
- Secondary matrices were spiked at low (2.00 ng/mL) and high (250 ng/mL) concentrations, 3 replicates each.
- Stored at freezer temperatures (-20 °C) for up to 60 days, analyzed and compared measured [Tl] to Day 0.
- Measured [Tl] at low concentration level compared to Day 0 samples ranged from 93.5-112%, RSD ≤ 7.3%.
- Measured [Tl] at high concentration level compared to Day 0 samples ranged from 99-111%, RSD ≤ 1.9%.

- **Method Validation Summary**
- Scientifically-defensible Tl concentration data can be collected in several biomatrices using this simple method.
- The method was successfully validated over the range 1.25-500 ng Tl/mL in rat plasma and other matrices based on linearity, recovery, precision, accuracy, instrument drift, and stability.

Validation Parameter	Experimental Design	Acceptance Criteria	Results
Linearity	8-pt matrix calibration curve (3 – 500 ng/mL)	$r \geq 0.99$ and %RE ≤ ±15% (≤ ±20% at LLOQ) using at least 6 points	**Passed**: $r \geq 0.99$ and %RE ≤ ±5.9% for all standards in Experiments 1, 2, and 3, using 8 points (1.25-500 ng/mL).
Lower Limit of Quant. (LLOQ)	Matrix standards in 6 replicates at 1.25 ng/mL	%RE ≤ ± 20% and %RSD ≤ 20%	**Passed**: %RE = -1.4% and %RSD = 1.0% at 1.25 ng/mL
Limit of Detection (LOD)	See LLOQ.	LOD = 3xSD for LLOQ replicates	**LOD = 0.0370 ng/mL.**
Selectivity	6 method blanks (with IS) and 6 matrix blanks (no IS)	Method blanks ≤ 30% of LLOQ response	**Passed**: Mean method blank response = 8.2% of 1.25 ng/mL response.
Absolute Recovery	Solvent standards prepared at same levels as matrix standards	Absolute recovery > 83% at each level, with variation ≤ 15% across the levels	**Passed**: Recovery 83-97%.
Intraday and Interday Precision & Accuracy	Triplicate matrix QCs at 3 levels; n=9 on each of 3 days	Mean RE ≤ ± 15% and RSD ≤ 15% (≤ ± 20% RE, RSD at LLOQ)	**Intra-day Passed**: Mean RE ≤ ±5.6 % and RSD ≤ 0.8%. **Inter-day Passed**: Mean RE ≤ ±4.8 % and RSD ≤ 4.3%.
Carryover	3 method blanks after high matrix standard	None; determine carryover	**Carryover present:** 1st blank <28%, 2nd and 3rd blank <5%
Dilution Verification	Triplicate matrix standards at ~1500 ng/mL; analyzed using a 150-µL injection (1:5 dilution)	Mean %RE ≤ ±20% and %RSD ≤ 20%	**Passed:** Mean %RE = -6.4% and %RSD = 5.4%.
Instrument Drift	Matrix standards run at start and end of sequence with multiple samples in between	%Diff ≤ ±15% (≤ ±20% at LLOQ) (second QC set vs. calibration set)	**Passed**: %Diff = -7.6 - -1.1%
IS Reproducibility	IS response in method blanks (intra-day) and QCs (inter-day)	None; determine IS reproducibility	**Intra-day:** %RSD ≤ 3.2% **Inter-day:** %RSD ≤ 4.3%.
Analyte Stability	Triplicate matrix standards at 3 levels; stored (A) on autosampler, (B) refrigerated extracts, (C) frozen samples, and (D) 3 freeze-thaw cycles of ≥ 24 hrs.	Mean % of Day 0 = 100 ± 20% and %RSD ≤ 20%	**Stable**: Mean % of Day 0 = 97.5 – 101% and %RSD ≤ 2.6%.
Secondary Matrix Precision & Accuracy	6 replicates at 2.00 ng/mL in each secondary matrix	Mean %RE ≤ ±15% and %RSD ≤ 15%	**Passed:** Mean %RE ≤ ± 14.1% and %RSD ≤ 1.5%.
Secondary Matrix Selectivity	6 method blanks (with IS) and 6 matrix blanks (no IS) in each secondary matrix	Method blanks ≤ 30% of LLOQ response	**Passed**: Mean method blank response ≤ 29.2 of LLOQ response.
Secondary Matrix Stability	3 replicates at low and high in each secondary matrix at 15, 30, and 60 days	Mean measured concentration 80-120% of Day 0 concentrations, RSD ≤ 20%	**Passed:** Mean [Tl] 93-112% of Day 0 and %RSD ≤ 7.3%.

Conclusions

- These results demonstrate a versatile, rugged analytical method with broad applicability to quantify total Tl in rat plasma and other matrices.

References

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