Supplementary Information

Determination of tantalum from tantalum oxide nanoparticle X-ray/CT contrast agents in rat tissues and bodily fluids by ICP-OES

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Experimental

Materials and reagents

Optima grade concentrated nitric (HNO_3) and hydrofluoric (HF) acids from Fisher Scientific (Pittsburgh, PA, USA) as well as Ultrex II ultrapure reagent hydrogen peroxide, 30%, (H_2O_2) and concentrated sulfuric acid (H_2SO_4) from J.T. Baker Avantor (Center Valley, PA, USA) were used throughout. All dilutions and solutions were prepared using deionized water (18.2 MΩ/cm) produced with a Milli-Q water purification system (Millipore, Bedford, MA, USA). Metals-free 15 and 50 mL polypropylene centrifuge tubes (VWR, Radnor, PA, USA) were used throughout.

Single-element 1000 µg/mL Ta was obtained from High-Purity Standards (Charleston, SC, USA) for the preparation of intermediate stock solutions of 50 and 1 µg/mL Ta in 1% HNO_3. The intermediate stock solutions and the 1000 µg/mL Ta stock standard were used to prepare 21 working calibration standards (0.001–40 µg/mL Ta) containing the acid matrix corresponding to the samples prepared by microwave digestion or open-beaker digestion methods. Twenty-three working calibration standards (0.001–100 µg/mL Ta) were prepared in the acid matrix corresponding to the samples prepared by the dry-ash digestion method. A 100 µg/mL Nb solution was prepared in 1% HNO_3 and 0.1% HF from a 10000 µg/mL Nb stock solution (High-Purity Standards) for use as an internal standard reference added to all sample dilution and working calibration solutions. Addition of the 100 µg/mL Nb solution was performed with an accurate and precise manual digital pipette; the volume added was 1 mL, 2 mL, or 5 mL for solutions diluted to a final volume of 10 mL, 20 mL, or 50 mL respectively. SPEX CertiPrep (Metuchen, NJ, USA) 10 µg/mL multi-element solution CLMS-4 and Inorganic Ventures (Christiansburg, VA, USA) 100 µg/mL multi-element solution CCS-5 control materials were used to verify the accuracy of the prepared calibration curves.

TaO NP agent solution digestion

Microwave-assisted acid digestion of Ta_2O_5 and NP agent stock. Tantalum (V) oxide powder (Ta_2O_5, Puratronic, 99.993% pure on metals basis excluding Nb, 50 ppm Nb maximum) purchased from Alfa Aesar (Ward Hill, MA, USA) was used as-received as a reference material for the determination of Ta content in CZ3-TaO NP agent solutions. NP agent stock (0.1 g) or Ta_2O_5 reference material (3–6 mg) was digested with 4 mL HF plus 1 mL HNO_3 in TFM vessels from CEM Corporation (Matthews, NC, USA) using a CEM Mars X-Press microwave digestion unit. The microwave digestion operating parameters were ramp to temperature (220 °C) in 25 min followed by a 30 min hold at temperature. The maximum permitted pressure was 600 psi and the maximum power output was 1600 W. The vessels cooled to less than 50 °C before depressurization. The dissolution solutions were transferred to polypropylene tubes and diluted to 50 g with deionized water. Four-fold secondary dilutions were prepared to contain 4% HF, 1% HNO_3, and 10 µg/mL Nb. Procedural blanks were carried through the digestion process alongside sample unknowns.

Acid dissolution of NP agent stock and dilute solutions. NP agent stock (0.1 g) was digested with 2 mL HF plus 0.5 mL HNO_3 in 50 mL polypropylene tubes using 1 hour of sonication at 50 °C (Branson Ultrasonic Cleaner 2510, Danbury, CT, USA). NP agent dilute solutions (0.2–2 g) were digested with 0.4 mL HF plus 0.1 mL HNO_3 in 15 mL polypropylene tubes following the same procedure as for agent stock. Niobium internal standard stock solution was added and the solutions diluted with deionized water to contain 4% HF, 1% HNO_3, and 10 µg/mL Nb. Procedural blanks were carried through the digestion process alongside sample unknowns.

Results and discussion

TaO NP agent solutions

In order to assign Ta concentration to the NP agent stock spiking solution, dissolution of the NP agent was conducted by microwave-assisted acid digestion alongside Ta_2O_5 reference powder. Reference material with certified Ta content that is of a similar matrix and properties as the TaO core of CZ NP agents is not available, so pure Ta_2O_5 powder was applied as the most suitable reference material available. Ta content in the reference powder was calculated from the trace-metals-basis purity provided on the certificate of analysis to be 81.89 w/w%. Although a certified Ta concentration is not provided for this material, its use as a reference material is preferable to running no reference material at all.[1] The Ta content in Ta_2O_5 reference powder was determined to be 81.5 ± 0.2 w/w% (n=6) with a relative percent recovery of 99.5 ± 0.3% (calculated by dividing the measured [Ta] by the theoretical [Ta] of 81.89% and multiplying by 100). Recovery of the reference material is considered sufficiently close to unity for the purposes of this study, establishing that the method is accurate for the quantitation of Ta from Ta_2O_5. NP agent stock solution was determined to be 1.730 ± 0.006 w/w% (n=3) by microwave-assisted acid digestion.

Using the microwave digestion method as the reference method,[1] the results assigned to the NP agent stock solution via the microwave digestion method were used to evaluate the accuracy a simpler acid dissolution method for determining Ta content in NP agent solutions. To that end, the same NP agent stock was prepared by acid dissolution with sonication and the result determined to be 1.736 ± 0.002 w/w% (n=3). Using the Ta content of 1.730 ± 0.006 w/w% assigned by microwave-assisted digestion as the reference value, the relative percent recovery of the mean Ta concentration obtained by acid dissolution calculated against the mean reference value obtained by microwave digestion is 100.3%. A two-sample t-test confirms that there is no statistical evidence that the means produced by the two methods are different. The acid dissolution procedure is preferred over microwave digestion for analysis of NP agent stock solutions because it is easier and faster to execute. Therefore, the Ta content in each NP agent dilute solution used in the reported spike recovery studies was determined by the acid dissolution procedure.

Biological spike recovery studies

Bias is expressed as relative spike recovery, *R*,

$$R = \frac{x' - x}{x_{spike}} \times 100$$

where x' is the measured result of the spiked sample, x is the inherent analyte concentration, and x_{spike} is added spike concentration.[2] Within-run recovery (*R*) and precision for each biological material per digestion method are provided in Tables S1-S3. Precision is calculated as the standard deviation of *R* from replicate digestions.

Table S1 Relative percent recovery (*R*) mean and standard deviation (SD) in blood, liver and femur by microwave digestion. Method 1a applied except where noted.

Sample	Run	n	Ta Spike[a] µg Ta	Ta Spike[a] µg Ta/g Sample	*R* ± SD %	*R* ± SD[c] %
Blood	1	3	5	25	99.6 ± 0.3	
	2	3	5	25	99.8 ± 0.8	
	3	3	5	25	99.8 ± 0.3	
	1-3	9	5	25		99.8 ± 0.5
	1	3	50	250	100.5 ± 0.2	
Liver	1	3	20	40	101.0 ± 0.7	
	2	3	20	40	100.0 ± 0.8	
	3	3	20	40	99.6 ± 0.6	
	1-3	9	20	40		100.2 ± 0.7
	1	3	610	1220	100.7 ± 0.7	
Femur	1	3	10	7	93.4 ± 2.7	
	2	3	10	7	89.1 ± 2.7	
	3	3	10	7	91.5 ± 1.8	
	1-3	9	10	7		91.3 ± 2.4
	4[b]	5	10	7	103.8 ± 2.2[b]	
	1	3	100	70	92.6 ± 0.4	

[a] Each replicate per run was spiked individually so the value listed is the mean spike per run.
[b] Femur Run 4 was conducted with the modified microwave digestion method 1b.
[c] Values listed are the mean *R* and pooled standard deviation of replicated runs.

Table S2 Relative percent recovery (*R*) mean and standard deviation (SD) in urine by open-beaker digestion (n=5)

Sample	Run	Ta Spike[a] µg Ta	Ta Spike[a] µg Ta/g Sample	*R* ± SD %	*R* ± SD[b] %
Urine	1	8670	867	99.3 ± 0.6	
	1	70100	7010	99.6 ± 0.6	
	2	70100	7010	97.2 ± 0.4	
	3	70100	7010	98.0 ± 0.4	
	1-3	70100	7010		98.3 ± 0.5

[a] Each replicate per run was spiked individually so the value listed is the mean spike per run.
[b] Values listed are the mean R and pooled standard deviation of replicated runs.

Table S3 Relative percent recovery (*R*) mean and standard deviation (SD) in carcass, feces and liver by dry-ash digestion

Sample	Run	n	Ta Spike[a] µg Ta	Ta Spike[a] µg Ta/g Sample	*R* ± SD %	*R* ± SD[b] %
Carcass	1	3	50	1	98.8 ± 3.8	
	2	3	50	1	96.1 ± 3.7	
	3	3	50	1	98.2 ± 2.2	
	1-3	9	50	1		97.7 ± 3.2
	1	3	1030	22	101.7 ± 0.4	
Feces	1	3	100	10	100.3 ± 2.5	
	1	3	3000	300	100.8 ± 0.7	
	2	3	3000	300	100.5 ± 0.3	
	3	3	3000	300	101.5 ± 0.1	
	1-3	9	3000	300		100.9 ± 0.4
Liver	1	3	20	2.5	102.1 ± 2.4	
	2	3	20	2.5	103.2 ± 1.9	
	1-2	6	20	2.5		102.7 ± 2.2
	2	3	1040	130	101.7 ± 1.1	

[a] Each replicate per run was spiked individually so the value listed is the mean spike per run.
[b] Values listed are the mean R and pooled standard deviation of replicated runs.

The purpose of developing multiple digestion approaches is to provide the analyst with options when processing the wide range of matrices and sample sizes presented for ICP analysis from an *in vivo* NP retention study. The digestion conditions have been refined empirically over the years into the current methods presented here; however, the methods could be more rigorously optimized according to the properties of each matrix (robust method optimization was not a goal of the present study). For example, we show that the recovery of Ta from femur is greatly dependent on when the internal standard is added during the digestion process and the conditions which resulted in good recovery of Ta from liver and blood did not work as well for femur. Initial results for the recovery of Ta from femur by method 1b are promising but it would be prudent to further refine the method for bone matrix.

For ease of handling and increased throughput, the authors prefer to apply microwave-assisted acid digestion over the other two methods. However, large samples, such as carcass and intestines, which are not easily homogenized for subsampling, must be processed in entirety. For this, our experience is that dry-ash digestion provides complete destruction of organic matter with minimal hands-on time, although the total processing time can take up to 24 hr per batch. In other cases, even for tissues which can be easily homogenized for subsampling, it is desirable to digest a whole organ if the suspected Ta content is near the method detection limit. For example, a whole rat liver can weigh around 10 g (wet-weight), so digesting the entire organ by dry-ashing versus a 0.5 g aliquot by microwave digestion results in an improved quantification limit of 10-fold (Table 4 of the manuscript). Digestion of urine by open-beaker (method 2) is a useful means to concentrate the 20 to 40 mL sample volumes sometimes submitted from long-term retention studies. However, microwave digestion would be a much more convenient approach for the digestion of urine when sample volumes are small or can be homogenized for effective subsampling. For this reason, the application of microwave digestion to urine samples for the quantitation of Ta resulting from excreted TaO NP warrants further investigation.

Method application – an *in vivo* retention case study

A seven-day retention study was conducted to evaluate the performance of a new TaO NP agent, CZ2, in terms of percent injected dose (% ID) of Ta retained. Portions of liver, kidney and spleen were processed by method 1a and the carcass was processed by method 3. Although control animals (i.e. injected with saline solution without TaO NP) were not included in this *in*

vivo study, procedural blanks were carried through the digestion and analysis process for each method alongside the tissues. The Ta concentration in the procedural blanks was determined to be less than the method limit of detection given in Table 4 of the main paper.

Mass of Ta measured per gram and per organ as well as calculated % ID retained are given in Table S4. Tissue Ta concentrations are calculated on a wet-weight or as-collected basis to remain consistent with the presentation of Ta concentration data in previous publications.[3] Clearance organs (liver, kidney and spleen) are evaluated individually for % ID retained (where Ta content is expected to be highest) in addition to total body retention, which is calculated by summing % ID retained within each clearance organ plus the remaining carcass. Sufficient sensitivity for the quantitation of Ta per organ is demonstrated by comparing measured µg Ta/g sample for liver, kidney, spleen and carcass in Table S4 to the LOD in Table 4 of the manuscript for the corresponding digestion method and sample size. We show that even the lowest Ta concentrations measured in the *in vivo* study range from 16- to 440-fold higher than the corresponding method LOD. Likewise, Ta concentrations in liver and spleen correlate to % ID retained values on the order of 20- and 300-fold lower than the critical level of 1.5% retained. The ability to quantitate % ID Ta retained at well below 1.5% for each organ allows us to demonstrate total body retention of less than 1.5% by the summation of the individual components. This is vital when correlating % ID retained to pathological response. Although low-level quantitation was the primary interest of the presented *in vivo* study, it is also required at times to accurately determine higher levels of Ta in tissues (e.g. as a result of a slow-clearing NP agent or to account for Ta in the excretion products where Ta could be relatively concentrated).[3a,3b]

Table S4 Seven-day retention study, 400 mg Ta/kg CZ2-TaO dose (~80 mg Ta injected per rat)

	µg Ta/g sample[a] measured	µg Ta per organ or carcass	% ID retained per organ or carcass	% ID retained body total
Rat 1				1.137
Liver	4.9	54.5	0.073	
Kidney	96.4	232.2	0.310	
Spleen	6.3	3.8	0.005	
Carcass	2.65	560.9	0.749	
Rat 2				1.212
Liver	7.1	65.2	0.080	
Kidney	124.0	254.3	0.313	
Spleen	8.9	4.5	0.005	
Carcass	3.45	662.0	0.814	
Rat 3				1.131
Liver	5.4	53.8	0.062	
Kidney	124.7	244.2	0.283	
Spleen	8.7	4.5	0.005	
Carcass	3.40	673.6	0.781	
Rat 4				1.024
Liver	5.2	59.3	0.068	
Kidney	98.6	215.1	0.246	
Spleen	8.4	4.2	0.005	
Carcass	3.36	617.0	0.705	

[a] Wet-weight of tissue at time of collection.

References for supplementary information

1 M. Thompson, S. L. R. Ellison and R. Wood, *Pure Appl. Chem.*, 2002, **74**, 835–855.

2 B. Magnusson and U. Örnemark (eds.), EURACHEM Guide, The Fitness for Purpose of Analytical Methods, 2nd edn, 2014.

3 (a) A. S. Torres, P. J. Bonitatibus, Jr., R. Colborn, G. D. Goddard, P. F. FitzGerald, B. D. Lee and M. E. Marino, *Invest. Radiol.*, 2012, **47**, 578–587; (b) P. J. Bonitatibus, Jr., A. S. Torres, B. Kandapallil, B. D. Lee, G. D, Goddard, R. E. Colborn and M. E. Marino, *ACS Nano*, 2012, **6**, 6650–6658; (c) P. J. Bonitatibus, Jr., A. S. Torres, G. D. Goddard, P. F. FitzGerald and A. M. Kulkarni, *Chem. Commun.*, 2010, **46**, 8956–8958; (d) P. J. Bonitatibus, Jr., M. D. Butts, R. E. Colborn, A. S. Torres, US Patent, 8,821,838 B2, 2014; (e) M. D. Butts, P. J. Bonitatibus, Jr., R. E. Colborn, A. S. Torres, US Patent, 8,728,440 B2, 2014.