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Determination of Ti from TiO_2 Nanoparticles in Biological Materials by Different ICP-MS Instruments: Method Validation and Applications

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Abstract

The accurate determination of nanoparticles (NPs) content in biological samples is of great interest for studying its potential impacts on health. This paper describes a method validation process for Ti determination from TiO_2 NPs in biological matrixes using a closed-vessel microwave digestion with a mixture of 3 mL of HNO_3 and 0.4 mL of HF and a detection by quadrupole inductively coupled plasma-mass spectrometry (Q-ICP-MS), in both standard and collision/reaction cell (CCT) modes. As no suitable certified reference materials are available, several internal reference materials (IRMs) were prepared precisely and they were used for the optimisation and the analytical quality assurance of the method. Several criteria such as linearity, limits of quantification (LOQ), precision under repeatability conditions and intermediate precision reproducibility were evaluated. Furthermore, the method was compared on different Q-ICP-MS devices and on a high resolution (HR)-ICP-MS using the prepared IRMs and various samples of rat tissues exposed to two different sizes of TiO_2 NPs. The results demonstrated that the Q-ICP-MS method evaluated permitted an accurate determination of Ti present as TiO_2 NPs in both standard and CCT modes, with a greater accuracy in CCT mode at low concentration levels near the LOQ of 0.10 mg kg^{-1}.

Keywords: Titanium; Engineered nanoparticles; ICP-MS; Method validation; Biological samples

Introduction

Nanotechnology is a new promising field with potential applications in domestic, industrial and biomedical products. Indeed, due to their physico-chemical characteristics as size, optical properties, elemental composition, material strength and catalytic activity, engineered nanoparticles (NPs) are used in the development of new applications in agriculture, engineering, processing, packaging, cosmetic and food sectors [1-5]. This expansion of nanotechnology applications leads to an increasing risk of human and environmental exposure to nanomaterials and to prompt discussions over the safety of these materials to human health. Some recent studies found that NPs are able to cross biological membranes and access organs, tissues and cells that larger particles normally cannot [6,7].

Among these NPs, titanium dioxide (TiO_2), a noncombustible and odorless white powder, naturally exists in three modifications (anatase, rutile and brookite). Nowadays, nano-sized TiO_2 is produced abundantly and used widely because of its thermodynamic stability, anticorrosion and photocatalysis e.g.as pigments in paints, toothpastes, plastics, paper, ceramics, cosmetics and additives in food (E171) [8,9]. Its use as food additives was restricted by the US Food and Drug Administration e.g. the quantity of TiO_2 does not exceed 1% by weight of the food. Studies on the potential toxicity of TiO_2 NPs (focused on mammals such as mice and rats) showed that TiO_2 NPs are able to accumulate in blood, liver and spleen [2,8,10-12]. Some TiO_2 nanoparticles have been shown to have inflammogenic, oxidative, and genotoxic effects [13] and the International Agency for Research on Cancer (IARC) classified TiO_2 as possibly carcinogenic to humans (Group 2B) [14].

Open hotplate [15,16] and microwave assisted digestion methods [17,18] with a mixture of nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) as reagents are widely used techniques for preparing solid samples for analysis of TiO_2 NPs in biological and food samples. Yet, the recent study of Weir et al. [19] indicated that microwave digestion using a mixture of HNO_3, H_2O_2 and hydrofluoric acid (HF) had higher reproducibility than hotplate digestion. Using ammonium persulfate as a fusing reagent, the fusion method developed by Khosravi et al. [20] achieved comparable recoveries rate with microwave digestion and higher than open hotplate digestion with a mixture of HNO_3, H_2O_2 and HF. Most of these studies used a quadrupole ICP-MS (Q-ICP-MS) detector for the quantification of TiO_2 NPs as Ti, in standard mode [10,15-19] or collision/reaction cell mode [20] to overcome Ti isobaric and polyatomic interferences. However, according to Sarmiento-Gonzalez et al. [17], interference attenuation using collision/reaction cell mode is insufficient to quantify Ti at the basal levels in complex samples and the authors used a high resolution ICP-MS (HR-ICP-MS) at medium resolution. In the context of the EU Joint Action Nanogenotox, Krystek et al. [21] organized an interlaboratory study (four labs) on the detection of Ti present as NPs in rat tissues with the use of different digestion methods and different ICP-MS (Q-ICP-MS or HR-ICP-MS) devices. Despite the differences in methodology used, the results obtained for samples with Ti concentrations >4 mg Ti kg^{-1} tissue were in agreement. However, in the case of Ti concentrations <4 mg Ti kg^{-1} tissue, the results obtained by Q-ICP-MS were probably biased by the presence of interferences. . Sarmiento-Gonzalez et al.

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[17] recommends to use an HR-ICP-MS device for Ti Determination. However, one could ask the question whether this really is necessary and whether there may be an alternative approach which could avoid use of the very expensive HR-ICP-MS technique.

To answer these questions, the aim of the present work was:

- To develop and validate an analytical method for Ti determination from TiO_2 NPs in biological samples by closed-vessel microwave digestion and Q-ICP-MS detection, using different internal reference materials (IRM) spiked with different dispersions of TiO_2 NPs, as no certified reference materials were available [22];

- To compare and evaluate the results obtained on different Q-ICP-MS devices with or without a collision / reaction cell and HR-ICP-MS.- to analyse several biological samples for the determination of tissue Ti levels after treatment of animals with the nanoform of TiO_2 in cases of an acute toxicity study for the EU Joint Action Nanogenotox.

Materials and Methods

Reagents and standards

All solutions were prepared with analytical reagent-grade chemicals and ultrapure water (18 MΩ cm) generated by purifying distilled water with the Milli-QTM PLUS system connected to an Elix 5 pre-system (Millipore SA, St. Quentin en Yvelines, France).

a. Hydrogen peroxide (H_2O_2, 30% (v/v); Normapur quality grade, nitric acid (HNO_3, 67% (v/v) and hydrofluoric acid (HF, 47% (v/v)); both Normatom quality grades, were purchased from VWR (Fontenay-sous-Bois, France).

b. Standard stock solutions containing 1000 mg L^{-1} of titanium (Ti) and internal standard solutions containing 1000 mg L^{-1} of gallium (Ga) and indium (In) were purchased from Analytika (Prague, Czech Republic) and were used to prepare calibration and internal standards. Working standards were prepared daily in 6% of HNO_3 and were used without further purification. A 10 mg L^{-1} multi-element solution (Agilent Technologies, Courtaboeuf, France) was used to prepare a 1 µg L^{-1} tuning solution containing several elements such as cobalt (Co), cerium (Ce), yttrium (Y) and lithium (Li), that allowed to sweep a wide range of mass. To obtain a linear response of the detector between pulsed and analogical modes, a 2.5 mg L^{-1} to 20 mg L^{-1} factor P/A multi-element solution (Agilent technologies) was used.

c. Lyophilized powder of Bovine Serum Albumin (BSA) with a purity ≥ 96% purchased from Sigma–Aldrich (St. Quentin Fallavier, France) and ethanol (EtOH) (purity 99.5%, VWR) were used in the TiO_2 NPs dispersion protocol.

d. Certified Reference Material (CRM): TM-15.2 from filtered Lake Ontario water was purchased from HORIBA Scientific (Longjumeau - France).

e. Titanium dioxide (TiO_2) material: Pure TiO_2 engineered nanoparticles (TiO_2 NPs): (Anatase, 32 nm) was purchased from VWR (Fontenay-sous-Bois, France); titania-based products included in the tested Nanogenotox materials were provided by the Joint Research Centre (JRC, Geel, Belgium) and are named according to their generic codes (NM-100, NM-101, NM-102, NM-103, NM-104, NM-105). Table 1 shows the characteristic of the selected TiO_2 NPs material.

f. Ultrapure grade carrier (argon (Ar) and helium (He), 99.9995% pure for each one) was supplied by Linde (Montereau, France).

Sample selection and preparation

TiO_2 engineered nanoparticles: Before preparation of IRMs, TiO_2 NPs dispersion was prepared using a 400 Watt Branson Sonifier S-450D equipped with a Cup-Horn (25 mm) (Branson Ultrasonics Corp, VWR, Strasbourg, France). The dispersion protocol used in this study was developed by the Work-Package 4 (WP4) of the Nanogenotox project [23], with two exceptions: (i) the use of a cup-horn instead of a disruptor horn made in Ti, and (ii) the increase of run time and amplitude from 16 min at 400 W and 10% to 20 min at 400 W and 20%, respectively, because the cup-horn is less efficient than the disruptor horn.

Briefly, approximately 15.36 mg of TiO_2 NPs was weighed in the glass scintillation vial (20 mL) in the presence of an Electrostatic neutralizer (Mettler Toledo, Viroflay, France) in order to remove the electrostatic charge on the vial. 30 µL EtOH was added drop-by-drop onto the particles in the vial by pipette. Then, 5.97 mL of 0.05% BSA water was added by pipette while slowly rotating and swirling the 45° tilted scintillation glass. The dispersion vial was closed and immerged in the cup-horn in the presence of ice-water to minimize heat development during sonication. Finally, sonication was applied for 20 min at 400 W and 20% of amplitude. The final theoretical concentration of TiO_2 NPs obtained was 2.56 g L^{-1}.

Organ samples from the Nanogenotox acute toxicity study: For the determination of tissue Ti levels after treatment with the nanoform of TiO_2, organ samples from animals were prepared by the National Institute for Public Health and the Environment (RIVM, The Netherlands) within the Nanogenotox Joint Action Plan. Six-week-old male Wistar rats (HsdCpb:WU) were used after intravenous administration of TiO_2 NM-100 and NM-102 in the acute toxicity study according to the Nanogenotox dispersion protocol [23]. The administered dose was 2.3 mg TiO_2 per animal, ranging from 8.6 to 10.3 mg TiO_2 kg^{-1} depending on the actual weight (range 224-266 g) of the animal. No sign of toxicity was observed and animals were autopsied at day 14 after exposure. 28 organs consisting of liver, lung, spleen, heart, kidney, brain and muscle were collected from the four animals, two animals (#2 and #3 represents male and female, respectively) treated with NM-100 and two animals (#12 and #13 represents male and female, respectively) treated with NM-102. Organs were homogenized by manual cutting and stirring, divided in several fractions and stored at -20°C.

Code-supplier	Product	Crystalline phase and reported size	Example of application
TiO_2-VWR	Ti (IV) oxide anatase	Anatase, 32 nm	Laboratory studies
NM-100	Tiona AT-1	Anatase, 200-220 nm, spherical	Pigment, paper, ceramics
NM-101	Hombikat UV 100	Anatase, 7-10 nm, spherical	Photocatalytic effects
NM-102	PC105	Anatase, 15-25 nm, spherical	Photocatalytic effects
NM-103	UV TITAN M262	Rutile, 20 nm, hydrophobic spherical	Cosmetics
NM-104	UV TITAN M212	Rutile , 20 nm, hydrophilic spherical	Cosmetics
NM-105	Aeroxide TiO_2 P-25	85% Anatase/15% Rutile, 22 nm, spherical	Photocatalytic effects

Table 1: Basic information of TiO_2 NPs.

Preparation of Internal Reference Materials (IRM): Due to the lack of suitable certified reference materials (CRM) containing TiO_2 NPs [22], five different fresh organs (beef kidney, calf heart, calfliver, calf muscle and calfbrain) were selected as test matrices to prepare IRM notably for the optimisation and validation processes, by adding TiO_2 NPs after dispersion. Each matrix differs from others in TiO_2 NPs properties and/or in the spiking concentration (Table 2). About 1 kg of samples was purchased from local markets. After washing with distilled water to remove foreign matters and dirt, the samples were prepared depending of the matrix. As example, for the liver, it was necessary to further treat the tissue samples to remove the blood-vessels. The samples were mixed until homogeneous matrix was obtained. Then, to disperse homogeneously TiO_2 NPs in the matrix, a previously prepared solution of TiO_2 NPs in dispersion (paragraph 2.2.1) were introduced in the IRMs. After spiking, portions of approximately 50 g of material obtained were distributed in polypropylene flasks (previously washed, soaked in the 10% (v/v) nitric acid, rinsed with deionized water and dried) and stored at -18°C.

The homogeneity study started after the end of sample preparation. Ten samples in duplicate were taken randomly from the prepared samples and analysed in both standard and He modes under repeatability conditions; details see 2.3 and 2.4. The degree of homogeneity of the test material was assessed statistically. The sampling and analytical procedures were in accordance with a recommended procedure [24]. The value of the inter-sampling standard deviation (σ_{sam}) was estimated from the mean squares after one-way analysis of variance (ANOVA), and a statistical test was carried out. This ISO/IUPAC/AOAC Harmonised Protocol for Proficiency Testing requires that σ_{sam} should be less than 30% of the target standard deviation σ_p, that is $\sigma_{sam} / \sigma_p < 0.3$ [25]. On three monthly basis, at least three samples were chosen randomly and analysed in duplicate under repeatability conditions for monitoring the stability of the analytes. The degree of stability of the test material was assessed statistically in accordance to ISO 13528 [25] that requires that the difference between a mean value of the samples to confirm homogeneity study (x) and stability study (y) for an absolute value should be less than 30% of the target standard deviation, that is $|x - y| < 0.3\ \sigma_p$. In all cases, the samples were statistically considered to have "sufficient homogeneity and stability" (Table 3).

IRM	TiO_2 NPs	*Th-V	**TV Std mode	**TV He mode
Calf heart	NM-105	0.170	0.243	0.147
Beef Kidney	TiO_2-VWR	1.26	1.34	1.28
Calf liver	TiO_2-VWR	3.80	3.47	3.52
Calf muscle	NM-102	4.19	3.88	3.86
Calf brain	NM-100	7.45	7.75	7.38

*Theoretical value spiked in MRI calculated considering [Ti]=60% [TiO_2]

**Target values in standard and in He modes, respectively

Table 2: Determination of IRMs target values by Q-ICP-MS Agilent 7700x in standard and He modes (in mg Ti kg^{-1}) (n=10).

Digestion procedure: Sample digestion was carried out using a Multiwave 3000 microwave digestion system (Anton-Paar, Courtaboeuf, France), equipped with a medium-throughput rotor with 16 medium-pressure vessels made of PEEK and PTFE-TFM (100 mL). In all experiments, the sample weight introduced in vessel varied between 0.3 and 0.4 g. After cooling at room temperature, the resulting digests were quantitatively transferred into 50 mL polyethylene flasks. 100 µL of the internal standard solution (2 mg L^{-1}) was added to achieve a final concentration of 4 µg L^{-1} and then the digested samples were completed with ultrapure water to the final volume before analysis by ICP-MS. The microwave program and the volumes of acids used were optimised for this study and described in section Optimisation of digestion procedure.

ICP-MS measurements

Instrumentation: ICP-MS measurements were mainly performed using an Agilent 7700x series instrument (Agilent Technologies, Courtaboeuf, France). The sample solutions were introduced in the instrument by a peristaltic pump from tubes arranged on a CETAC ASX 500 Model 510 auto sampler (CETAC, Omaha, Nebraska, USA). This device was equipped with a third generation collision/reaction cell, the Octopole Reaction System (ORS^3), which works using He gas (hereafter abbreviated as He mode).

For comparison, three other ICP-MS devices were used: two Q-ICP-MS (XSeries 2, iCAP Q) and an HR-ICP-MS (ELEMENT 2)

Optimisation: Torch position, ion lenses and gases output were optimized daily with the 1 µg L^{-1} tuning solution to carry out a short-term stability test of the instrument, to maximize ion signals and to check the background level and minimize interference effects caused by polyatomic ions (oxide levels CeO^+/Ce^+ <1.2%) and doubly charged ions (Ce^{2+}/Ce^+ <2%). Linearity response in the pulsed and in the analogical mode (P/A factor determination) was daily verified using PA tuning solutions. Further details of the instrument settings and data acquisition parameters are given in Table 4.

Calculations and statistical methods: Concentrations were expressed in mg of Ti per kg fresh material. The Ti from TiO_2 NPs was analyzed considering [Ti]=60% [TiO_2].

The optimised method was validated according to the accuracy profile procedure [26,27], based on tolerance intervals to select the best calibration function and to determine the validated concentration ranges. Accuracy profile summarizes in one plot every validation

IRM	Analyse date	Mean (mg kg^{-1})	σ_{sam}	σ_p	Homogeneity (σ_{sam}/σ_p)*	Stability ($\|X-Y\|/\sigma p$)*
Calf heart	(X, n=20): 23/08/2012 (Y, n=06): 16/11/2012	0.138 0.147	0.004	0.030	0.13	0.29
Beef Kidney	(X, n=20): 02/08/2012 (Y, n=06): 16/11/2012	1.24 1.28	0.048	0.192	0.25	0.23
Calf liver	(X, n=20): 03/08/2012 (Y, n=06): 16/11/2012	3.41 3.52	0.031	0.454	0.07	0.24
Calf muscle	(X, n=20): 24/08/2012 (Y, n=06): 16/11/2012	3.88 3.86	0.030	0.869	0.03	0.02
Calf brain	(X, n=20): 23/08/2012 (Y, n=06): 16/11/2012	7.14 7.38	0.059	0.850	0.07	0.27

*Critical value > 0.3

Table 3: Homogeneity and stability study for TiO_2 NPs in IRMs, analyse performed by ICP-MS Agilent 7700x with He mode.

element, giving a graphical representation of the error risk for each concentration on the validated range. This procedure gives a precise estimation of the accuracy of the analytical method and determines the limit of quantification (LOQ) according to the maximum error risk accepted. The accuracy expresses the total error including the systematic error (trueness) and the random error (repeatability and intermediate precision) for each concentration level. The validity domain can be defined between the limit of quantification and the upper tested concentration, as the β-expectation limits are comprised between the acceptance limits. The LOQ is deduced from the intersection between acceptability and tolerance limits. Tolerance limits (β-expectation tolerance interval) were calculated for the mean bias at each concentration level.

Results and Discussion

The element Ti consists of five naturally abundant isotopes (46Ti (8.0%), 47Ti (7.3%), 48Ti (73.8%), 49Ti (5.5%) and 50Ti (5.4%). Yet, 46Ti, 48Ti and 50Ti isotopes cannot be used for quantification due to strong isobaric and polyatomic interferences. A preliminary study conducted on the two other Ti isotopes on deionised water and biological samples using different spiked solutions with Ti (2, 5, 10, 20 and 50 µg L^{-1}) and different non target elements multi-elemental solution (10, 20, 40, 80 and 160 µg L^{-1} of Li, Al, Cr, V, Ni, Zn, Co, Cu, Ge, Se, Sc, Mn, Fe, Sr, Mo, Y and 0.5, 1 and 5 mg L^{-1} of Ca, K, Mg, Na) showed that 47Ti and show the least interference in biological samples (see supplementary data). 71Ga and 115In were tested as internal standards (IS) and both can be used for this application. So, for the remainder of this study, 47Ti, 49Ti and 71Ga as internal standard (4 µg L^{-1}) were used.

Optimisation of digestion procedure

First, based on a previous optimised microwave digestion procedure using 3 mL of HNO_3 [28], and on previous studies using microwave digestion and a mixture of HNO_3 and H_2O_2 [17,18] different mixtures of HNO_3 (3 mL) and H_2O_2 (0-3 mL) were tested. The addition of H_2O_2 did not improve the low recovery rates (Figures 1 and 2 without HF). So, different volumes of HF acid from 0.1 to 0.8 mL were added to the 3 mL HNO_3 to improve the solubilisation of TiO_2 NPs by a complexation process [29]. Results on a pure TiO_2 NPs (VWR, 32 nm) using a maximum power of 1400 W showed that a satisfactory dissolution of Ti from TiO_2 NPs was obtained with addition of at least 0.4 mL of HF acid (Figure 1). Consequently, a mixture of 3 mL HNO_3 and 0.4 mL HF

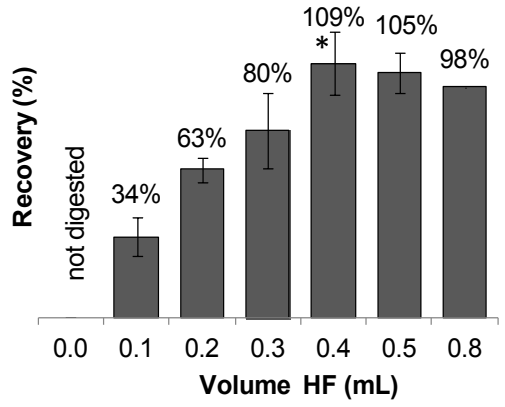

Figure 1: Recovery (%) according to the HF acid volume (mL).

(*) mean ± standard deviations (n ≥ 3)

was retained to optimise the microwave power between 1000 and 1400 W on the different pure NPs and on the beef kidney and calf liver IRMs (Tables 1 and 2). Examples of results with and without addition of HF (except for pure NPs) are presented in Figure 2A. With the addition of HF acid, all samples tested were fully digested and a satisfactory recovery was obtained when the applied power is greater than or equal to 1100 W. It should be noted that recovery extraction of TiO_2 NPs higher than 80% is also achievable without addition of HF and a power equal or above 1100 W but only for the low concentration (1.26 mg Ti kg^{-1}, Table 2) investigated (Figure 2B). On higher concentration (3.8 mg Ti kg^{-1}, see Table 2), the extraction of Ti without HF acid becomes limited (Figure 2C). Finally, a mixture of 3 mL of HNO_3 and 0.4 mL of HF and a microwave programme consisting of a power ramp from 500 to 800 W over 10 min, then from 800 to 1200 W over 10 min and held constant for 10 min were selected and used for the remainder of the study.

Comparison of IRM results on different ICP-MS devices

To evaluate the potential limitation of the use of Q-ICP-MS for Ti quantification in biological matrices, the IRMs were analysed before and after spiking NPs on three other ICP-MS instruments, two Q-ICP-MS (XSeries 2, iCAP Q) and an HR-ICP-MS (ELEMENT 2), all from Thermo Fisher Scientific, Courtaboeuf, France, (Table 4). The LOQs were found to be similar for the iCAP Q ICP-MS (0.10 mg kg^{-1}) in the MR and HR modes for ELEMENT 2. Ti target values obtained by the XSeries 2 were higher than the theoretical spiked Ti concentration. For all matrices with low Ti concentrations, the Ti measured by Q-ICP-MS XSeries 2 (standard mode) was interfered, unlike the other ICP-MS instruments used. However, the interference effect observed with XSeries 2 instrument becomes negligible at high Ti concentrations in liver, muscle and brain tissues (spiked values 3.8, 4.2 and 7.5 mg kg^{-1} respectively). In general, no interference was observed regardless of the mode used (with or without a collision cell) with Q-ICP-MS Agilent 7700x and Thermo Fisher Scientific iCAP Q instruments, and the results were close to the theoretical spiked Ti values and sufficiently in agreement with those obtained by the HR-ICP-MS instrument (recoveries generally varied between 80 and 113%, Table 6), except on the less concentrated heart tissue sample in standard mode (recoveries of 134% and 143% for iCAP Q and 7700x instruments, respectively). This can be attributed to the presence of low polyatomic interferences (between the LOQ and 2 × the LOQ) that can be eliminated by a collision cell (recoveries of 86 and 99%) or HR-ICP-MS instrument (recovery of 108% in MR mode).

This information indicates that Ti can be quantified in biological samples using Q-ICP-MS in standard mode, except below 0.20 mg kg^{-1} where the use of Q-ICP-MS instrument with a collision cell or HR-ICP-MS instrument becomes necessary. Finally, it should be noted that the subtraction of the unspiked values from the spiked values resulted in comparable Ti concentrations for all tested ICP-MS instruments and all analysis modes. In this case, the results were close to the theoretical spiked Ti values (recovery generally varied between 80 and 113%, except for heart tissue spiked at twice the LOQ with iCAP Q instrument in standard mode (75%)).

Method validation

Before validation with the accuracy profile procedure, the linearity and the limits of detection (LOD) and LOQ were evaluated. These measurements were carried out with the Agilent 7700x ICP-MS. Linearity experiments (n=11) were performed by using standard solutions at five concentration levels (0, 1, 5, 10, 20 and 30 µg L^{-1}).

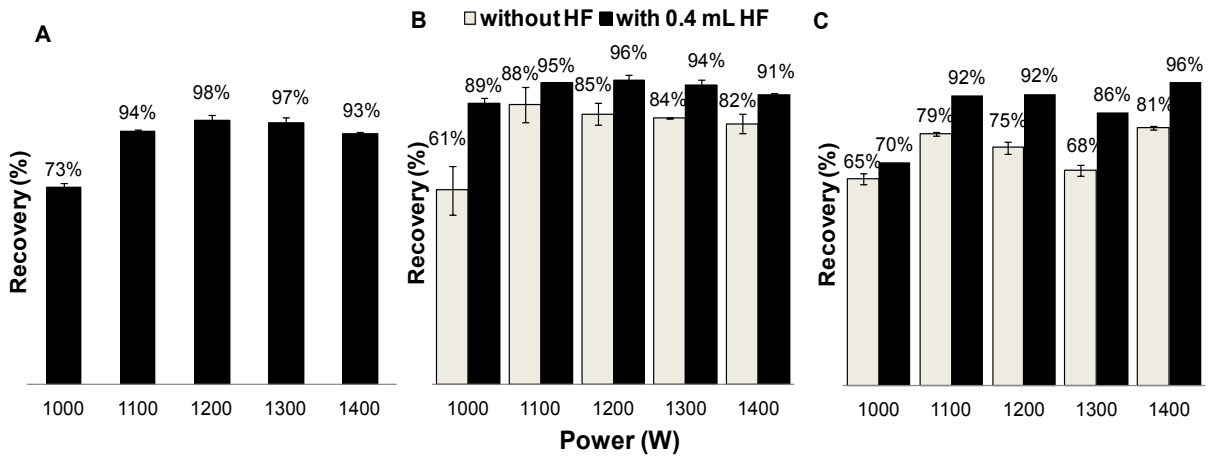

Figure 2: Optimisation of the power (W) applied for the digestion programme.

(A) Pure TiO_2 NPs (VWR, 32 nm), (B) beef kidney IRM, and (C) calf liver IRM, recoveries calculated using target values showed in Table 2

*mean ± standard deviations (n=3)

ICP-MS parameters	7700x (Agilent)	XSeries 2 (Thermo)	iCAP Q (Thermo)	ELEMENT 2 (Thermo)
Nebuliser	Concentric type 400 µL min^{-1}	Concentric type 900 µL min^{-1}	Concentric type < 200 µL min^{-1}	Concentric type < 200 µL min^{-1}
Spray chamber	Scott-type double-pass water cooled	Cyclonic in PTFE (Kit-HF)	Peltier-cooled cyclonic	Peltier-cooled cyclonic
Sampling/skimmer cones	Nickel	Nickel	Nickel	Nickel
Power	1400 W	1400 W	1550 W	1300 W
Plasma argon flow rate	15 L min^{-1}	13 L min^{-1}	14 L min^{-1}	16 L min^{-1}
Nebuliser argon flow rate	0.9-1 mL min^{-1}	0.95-1.1 mL/min	1 mL min^{-1}	1.3 mL min^{-1}
Auxiliary argon flow rate	0.99 L min^{-1}	0.80-0.90 L min^{-1}	0.79 L min^{-1}	0.88 L min^{-1}
He gas flow (collision cell)	4.3 mL min^{-1}	-	4.2 mL min^{-1}	-
Replicates	3	3	3	3

Table 4: Operating conditions of the four ICP-MS devices.

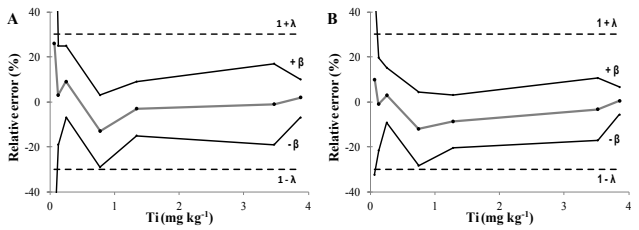

Figure 3: Accuracy profile (A) in standard mode, β=90%, λ (acceptability limits)= ± 30%; (B) in Hemode, β=80%, λ=± 30%.

Statistical tests based on application of analysis of variance to the least-square regression indicated that the linear regression model was acceptable and no deviation from the regression model was observed in the defined range (data not shown). The LOD and LOQ were defined respectively as three and six times the standard deviation of the average after correction for typical sample weight (0.3 g) and a final volume of 50 mL. Results of 21 reagent blanks analyzed indicated a LOQ of about 0.78 µg L^{-1} corresponding to 0.13 mg kg^{-1} in both standard and He modes.

The accuracy profile was built over a range of concentrations levels varied from the estimated LOQ and below the maximum of the external calibration curve (validity domain). The probability β was set to 90% and 80% in standard and in He mode, respectively, which means that the risk that future expected results will fall outside these limits is below 10% and 20% on average. The acceptance limits were set to ± 30% in both standard and He modes. In this study, the accuracy profile was built on seven concentration levels: spiked heart at ½ × LOQ, LOQ and 2×LOQ; brain IRM tenfold diluted; liver, kidney and muscle IRMs, corresponding to 0.06, 0.13, 0.26, 0.77, 1.3, 3.5, and 3.9 mg (Ti) kg^{-1}, respectively. All selected samples were digested and analysed in duplicate following the optimised method. This validation series was repeated on five different days over a period of three months by varying two operators. Accuracy profiles obtained in standard and He modes were presented in Figure 3. The performance characteristics calculated and presented in Table 5 are the trueness and the precision, the last one including the repeatability and the intermediate precision. The lowest concentration (½×LOQ) has to be excluded because its accuracy and intermediate precision were not satisfactory. All the β-expectation tolerance intervals were comprised within the acceptability limits between the LOQ and the highest level. The trueness bias (absolute value) ranged from 1.1% to 13% in the standard mode and from 0.3% to 12% in He mode. The estimated repeatability coefficient of variation (CV_r) varied from 3.9% to 8.3% in the standard mode and from 4% to 11.5% in the He mode. The obtained intermediate precision coefficient

Concentration (mg kg^{-1})	Accuracy					
			Precision			
	Trueness (relative bias %)		Repeatability (% RSD)		Intermediate precision (% RSD)	
	Std	He	Std	He	Std	He
0.06 (½ LOQ)	26	9.9	36	26	38	26
0.13 (LOQ)	2.8	-1.0	8.0	11	11	14
0.26 (2x LOQ)	9.0	3.0	3.9	8.2	7.1	8.2
0.747	-13	-12	7.0	5.5	9.3	12
1.3	-3.2	-8.7	5.0	5.1	6.4	8.3
3.5	-1.1	-3.3	8.3	9.3	9.6	9.8
3.9	1.7	0.3	4.5	4.0	4.5	4.2

Table 5: Comparison of performance characteristics achieved in standard and He modes.

of variation (CV_R) varied from 4.5% to 11% in the standard mode and 4.2% to 14% in the He mode.

The LOQ can be deduced from the intersection of the tolerance intervals and the acceptability limits. Note that the tolerance intervals may be asymmetrical when a significant bias is observed. In this case, the most pessimistic interval is selected [27,30]. For a 0.3 g sample weight and 50 mL dilution, LOQs were estimated at 0.12 and 0.10 mg (Ti) kg^{-1} in standard and in He modes, respectively. So, the analytical method can be declared as valid between 0.12 or 0.10 and 3.9 mg (Ti) kg^{-1} in standard and He modes, respectively.

For routine analysis, the CV_R was fixed at 12% for a mean value M < 2xLOQ and 10% for $M \geq$ 2xLOQ in standard mode, and at 15% for M < 2xLOQ and 12% for $M \geq$ 2xLOQ in He mode (Table 5). Using these CV_R values, the uncertainty of each result was defined as:

$$U = \frac{k \times CV_R \times M}{100 \times \sqrt{n}}$$

with k=2 (p=95%), M the mean value, and n the number of independent replicates. As example, for $M \geq$ 2xLOQ in standard mode for duplicates, the uncertainty was U=0.141xM.

Applications

Quality assurance: In the case of routine analysis, as part of the adopted internal quality control (IQC) procedure, an analytical sequence included: a reagent blank to check the absence of any contamination; a middle range standard to monitor instrumental drift every seven samples and at the end of the experiment and two IRMs (liver and kidney) to check the accuracy. Moreover, a certified water TM-15.2 from filtered Lake Ontario water containing (14.6 ± 1.3) µg Ti L^{-1} was also used but it was not digested and directly analyzed by ICP-MS. In this study, all the blanks were below the LOQ, no instrumental drift higher than 20% was observed and results of the IRMs and TM-15.2 samples were included in the confidence interval of 80-120% and considered satisfactory.

Analysis of biological samples and comparison of results: The Ti concentrations (in mg Ti kg^{-1}) determined by the four ICP-MS instruments in tissues from rats are summarized in Table 7. Results showed that liver and spleen are the most highly targeted tissues, with Ti concentrations ranging from 46 to 163 mg kg^{-1} followed by lung tissue (range 4.1-17.0 mg kg^{-1}). On these matrices, regardless of the ICP-MS instrument tested or the mode used, all results were comparable confirming that the interference effect is negligible for high concentration levels.

Concerning the others organs (heart, kidney, brain, and muscle), Ti concentrations varied between 1 to 2 times the LOQ. Results

IRMs	Mode	Spiked sample	Control sample	(S-C)*	Recovery**
Calf Heart (0.170 mg kg^{-1})					
7700x	Std	0.243	< 0.12	0.243	143%
	He	0.147	< 0.10	0.147	86%
XSeries 2	Std	0.645	0.509	0.136	80%
iCAP Q	Std	0.227	< 0.12	0.227	134%
	He	0.168	< 0.10	0.168	99%
ELEMENT 2	MR***	0.184	< 0.10	0.184	108%
	HR	0.222	< 0.10	0.222	131%
Bovine kidney (1.26 mg kg^{-1})					
7700x	Std	1.34	0.12	1.22	97%
	He	1.28	< 0.10	1.28	102%
XSeries 2	Std	2.31	1.19	1.12	89%
iCAP Q	Std	1.34	< 0.12	1.34	106%
	He	1.28	<0.10	1.32	105%
ELEMENT 2	MR	1.31	<0.10	1.31	104%
	HR	1.80	<0.10	1.18	94%
Calf liver (3.80 mg kg^{-1})					
7700x	Std	3.47	0.20	3.27	86%
	He	3.52	<0.10	3.52	93%
XSeries 2	Std	4.22	0.89	3.32	88%
iCAP Q	Std	3.60	0.21	3.39	89%
	He	3.53	0.13	3.40	89%
ELEMENT 2	MR	3.64	0.19	3.44	91%
	HR	3.95	<0.10	3.95	104%
Calf muscle (4.19 mg kg^{-1})					
7700x	Std	3.88	0.12	3.76	90%
	He	3.86	<0.10	3.86	92%
XSeries 2	Std	4.40	0.52	3.88	93%
iCAP Q	Std	4.11	< 0.12	4.11	98%
	He	4.18	<0.10	4.18	100%
ELEMENT 2	MR	4.06	<0.10	4.06	97%
	HR	4.11	<0.10	4.11	98%
Calf Brain (7.45 mg kg^{-1})					
7700x	Std	7.75	<0.12	7.75	104%
	He	7.38	<0.10	7.38	100%
XSeries 2	Std	7.57	0.42	7.14	96%
iCAP Q	Std	7.68	<0.12	7.68	103%
	He	7.74	<0.10	7.94	107%
ELEMENT 2	MR	7.76	<0.10	7.76	104%
	HR	8.40	<0.10	8.40	113%

*(S-C) is the subtraction of the control from the spiked values. No subtraction made if the Ti concentration in control samples (C) <LOQ

**Recovery = $\frac{[Ti]_{S-C}}{[Ti]_{Theoretical}}$

***MR: Medium resolution; HR: High resolution

Table 6: Ti measured in IRMs by four ICP-MS systems (in mg kg^{-1}).

	XSeries 2	7700x		iCAP Q		ELEMENT 2	
Samples	**Std**	**Std**	**He**	**Std**	**He**	**MR**	**HR**
Rats treated with NM-100 TiO_2							
Liver #2	120	113	122	121	117	118	121
#3	120	103	109	103	99	104	99
Lung #2	8.2	6.2	7.0	7.0	7.2	7.5	6.8
#3	5.5	4.1	4.4	4.5	4.4	4.5	4.6
Spleen #2	155	141	148	153	156	158	163
#3	136	125	124	130	130	133	129
Heart #2	1.4	0.27	0.13	0.26	0.12	0.13	<0.10
#3	1.6	0.20	0.19	0.26	<0.10	0.29	<0.10
Kidney #2	1.3	0.13	<0.10	0.17	<0.10	<0.10	<0.10
#3	1.0	<0.12	<0.10	0.16	<0.10	<0.10	<0.10
Brain #2	0.9	<0.12	<0.10	<0.12	<0.10	<0.10	<0.10
#3	0.6	<0.12	<0.10	<0.12	<0.10	<0.10	<0.10
Muscle #2	1.2	0.12	<0.10	0.24	<0.10	<0.10	<0.10
#3	1.0	<0.12	<0.10	0.19	<0.10	<0.10	<0.10
Rats treated with NM-102 TiO_2							
Liver #12	120	111	114	115	112	116	112
#13	120	108	110	113	114	111	115
Lung #12	17	15	14	16	15	16	16
#13	15	12	13	14	15	14	14
Spleen #12	65	58	60	63	63	64	66
#13	53	46	49	47	48	49	46
Heart #12	1.3	0.17	0.21	0.25	<0.10	<0.10	<0.10
#13	1.5	0.25	0.12	na	na	na	na
Kidney #12	1.0	0.12	<0.10	0.23	<0.10	<0.10	<0.10
#13	1.0	<0.12	<0.10	na	na	na	na
Brain #12	0.7	<0.12	<0.10	0.19	<0.10	<0.10	<0.10
#13	0.7	<0.12	<0.10	na	na	na	na
Muscle #12	1.4	0.43	0.39	0.64	0.50	0.54	0.42
#13	1.0	<0.12	<0.10	na	na	na	na

na: not analysed

Table 7: Ti concentration (mg kg^{-1}) in various organs issued from rats exposed to TiO_2 NPs determined by four ICP-MS systems.

obtained on Q-ICP-MS instruments (Agilent 7700x and iCAP-Q) and HR-ICP-MS (Element 2) were similar, but it should be noted that the use of a collision cell (He mode) or high resolution slightly improved Ti determination in these organs. This confirms that a slight interference effect was present in the standard mode analysis at low concentration levels (between 1 to 2 times the LOQ). However, the XSeries 2 Q-ICP-MS instrument presents a very high isobaric interference at these low concentrations (1 mg kg^{-1} instead of 0.1 to 0.2 mg kg^{-1} obtained by the three other ICP-MS instruments). These comparisons between different ICP-MS instruments at high and at low Ti concentrations confirm our study conducted on IRMs where it was observed that the Ti is not interfered with when using a more recent Q-ICP-MS instrument (Agilent 7700x and iCAP Q) or an HR-ICP-MS instrument directly.

Conclusions

Using five internal reference materials, a comparison study between different ICP-MS instrumentsshowed that unlike the old Q-ICP-MS instrument generation (e.g. XSeries 2), the analysis of Ti by a more recent generation of Q-ICP-MS instruments (i.e. 7700x and iCAP Q) shows negligibly interferencewith in standard mode and no interference with using the CCT mode. Results obtained by the most recent generation of Q-ICP-MS instrument and by HR-ICP-MS instrument were similar and consequently offer the scientific community a less expensive alternative to HR-ICP-MS technology. Consequently, the method was validated on a recent Q-ICP-MS instrument (7700x), using a mixture of HNO_3/HF, with microwave digestion in a closed-vessel for sample preparation. The criteria of performance demonstrated that the method enabled accurate determination of the Ti present as TiO_2 NMs in biological samples in both standard and CCT modes, with greater accuracy in CCT mode at concentration levels near the LOQ of 0.10 mg kg^{-1}.

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