

ICP-OES Analysis of Titanium Dioxide in Foods Prepared with a One-Step Digestion

Robust analysis of difficult food matrices using the Agilent 5800 ICP-OES with smart tools



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Introduction

Titanium dioxide (TiO_2) has been used in a diverse range of industries since it was first discovered in 1791. In more recent times, TiO_2 has been added to cosmetics, hygiene products, paints, pharmaceuticals, and foods (1), primarily as a coloring (whitening) agent (2).

However, the European Union (EU) has raised health concerns relating to the consumption of TiO_2 nanoparticles (NPs) present in food products that contain TiO_2 . NPs are defined as constituent particles with an external diameter less than 100 nm (3). According to the European Food Safety Authority (EFSA) panel on Food Additives and Flavorings (FAF), concerns for genotoxicity following the consumption of TiO_2 cannot be ruled out. Given these uncertainties, the panel concluded that the food additive TiO_2 (E171) could no longer be considered as safe (4, 5). The European Commission (EC) proposal to ban the use of E171 as a food additive from 2022 was approved by state members on October 8, 2021 (5).

EFSA hasn't specified maximum detectable limits of TiO₂ in food products as of June 2023. However, China's GB5009.268-2016 Determination of Multiple Elements in Food specifies the measurement of 16 elements—including Ti—by ICP-OES. If the EFSA regulations are similar to GB5009.268-2016, an ICP-OES that can achieve low detection limits and minimize food matrix-based interferences will be needed.

To meet the requirements for the application, the Agilent 5800 Vertical Dual View (VDV) ICP-OES with Agilent ICP Expert Pro software includes several smart tools to assist the analyst. The following tools aid method development, ensure data quality, and optimize speed of analysis, making the 5800 ICP-OES and Agilent 5900 ICP-OES the most suitable instruments for analysis of TiO₂ in food:

- IntelliQuant Screening assists method development by identifying and semi quantifying up to 70 elements in different food samples with varying matrices. IntelliQuant can also be used in routine quantitative analysis, providing a quality control (QC) check on samples, to check for spectral interferences, and provide sample insight to ensure accuracy throughout the analysis.
- Outlier Conditional Formatting (OCF) helps with the easy identification of QC failures, overrange results, or poor precision (RSDs). Without OCF, these problems can be easily missed, leading to poor recoveries and unexpected results. OCF helps to identify problematic data points within large data sets in real-time, saving review time post-analysis and improving the quality of the quantitative results.
- Intelligent Rinse maximizes throughput and reduces sample-to-sample contamination by optimizing rinse times between samples. Intelligent Rinse monitors intensities of nominated element wavelengths during the rinse period and automatically ends the rinse when these intensities reach a user-specified threshold.

In this study, the 5800 VDV ICP-OES was used to measure TiO₂ as Ti, plus arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), silicon (Si), and zinc (Zn) in a range of food products. The foods included confectionary, dessert decorations, a salad dressing, and a protein bar. All foods were prepared using a simple one-step microwave digestion procedure using sulfuric acid and hydrogen peroxide. The absence of hydrofluoric acid (HF) from the digestion resulted in a simplified and safer sample preparation method.

Experimental

Instrumentation

All data was collected using the 5800 VDV ICP-OES configured with an Agilent SPS 4 autosampler. The sample introduction system consisted of a SeaSpray glass concentric nebulizer, double-pass cyclonic spray chamber, and an Agilent Easy-fit semi-demountable VDV torch with a 1.8 mm inner diameter. The instrument and method parameters were set up, optimized, and controlled using ICP Expert Pro software (Table 1).

The Intelligent Rinse function within the software monitors the intensities of nominated element wavelengths during the rinse period and controls the rinse times (6). Intelligent Rinse automatically ends the rinse when element intensities reach a user-specified washout threshold of 'quick', 'moderate', or 'thorough'. To minimize run-to-run contamination throughout this study, *thorough* Intelligent Rinse mode was selected.

Table 1. Agilent 5800 VDV ICP-OES instrument and method parameters.

Parameter	Setting
Read Time (s)	10
Replicates	3
Sample Uptake Delay (s)	20
Stabilization Time (s)	15
Intelligent Rinse	Enabled (Thorough)
Pump Speed (rpm)	12
Fast Pump	Enabled
RF Power (kW)	1.4
Auxiliary Flow (L/min)	1.0
Plasma Flow (L/min)	12.0
Nebulizer Flow (L/min)	0.7
Sample Pump Tubing	White-white
Waste Pump Tubing	Blue-blue
Background Correction	FBC and FACT
View Mode	Axial

Sample preparation

To evaluate the digestion procedure and analytical method's capacity to measure Ti in complex matrices, a variety of food samples that contained TiO₂ were bought from a local supermarket in Mulgrave, Australia. The samples included a packet of confectionery, a decorative 'gold dust' spray, salad cream, and a protein bar.

To verify the method, NIST 1566b Oyster Tissue standard reference material (SRM, National Institute of Standards and Technology, Gaithersburg, US) and NIES No. 9 Sargasso certified reference material (CRM, National Institute for Environmental Studies, Ibaraki, Japan) were used. All samples and CRMs were prepared by microwave digestion using the same conditions within the Mars 6 microwave digestion system (CEM, Matthews, NC, USA).

The foods and CRMs were digested in concentrated sulfuric acid (H₂SO₄, ACS reagent, 95.0–98.0%, Sigma-Aldrich) and hydrogen peroxide (H₂O₂, 30%, Analytical Grade, Merck). 500 mg of each sample was digested with 9 mL of H₂SO₄ and 1 mL of H₂O₂ at a temperature of 250 °C for 1 h using the program outlined in Table 2. The digested solutions were diluted with Milli-Q water to a final volume of 50 mL. The solutions were then centrifuged at 3000 RPM in a 5702 centrifuge (Eppendorf, Hamburg, Germany) to pellet the particulates. The supernatant was transferred to a clean tube before analysis by the 5800 ICP-OES. All samples were prepared in triplicate.

Table 2. Microwave digestion parameters used for all food samples.

Parameter	Setting
Maximum Power (W)	1800
Temperature (°C)	250
Ramp Time (min)	25
Hold Time (min)	60

Method development

IntelliQuant Screening was used to speed up method development by recommending the best analyte wavelengths to use for the analysis of the food sample digests. The results also provided estimated concentrations of the elements in the samples, helping to determine the appropriate working calibration range for each analyte.

To aid interpretation of the semiquantitative data, the IntelliQuant results can be presented visually, including as a periodic table 'heat map', a pie chart, or a bar chart. The periodic table heat map of the decorative gold dust spray sample (Figure 1) shows a high concentration of Fe. Fe can cause interferences on analytes such as Cd, potentially leading to false positive readings, as flagged by ICP Experts' star ranking on the Cd 226.502 nm analyte wavelength. The analyst can use the insights provided by the IntelliQuant Screening data when considering background correction, as shown in Figure 3.

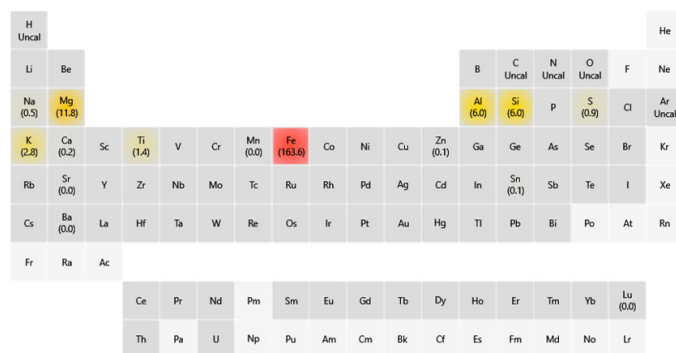


Figure 1. IntelliQuant Screening periodic table 'heat map' view showing which elements are present in the decorative spray sample. All values are semiquantitative and are reported in ppm.

As high concentrations of H₂SO₄ were used during sample preparation of the food samples, high levels of instrument robustness and matrix tolerance were required for the analysis. Also, consideration was needed to account for the fact that the Ti emission intensity can be suppressed by H₂SO₄ concentrations as low as 5% (2). As Ti was the primary target analyte of this work, it was essential to matrix-match all H₂SO₄ concentrations across all samples to ensure data accuracy. Standards were also matrix matched to ensure identical nebulization efficiency throughout the analytical run and from sample to sample.

Typically, one rinse time is selected between sample runs that will suit the highest concentration samples or to wash out samples with complex matrices. With the Intelligent Rinse function, however, rinse times between samples can be optimized automatically by the software. Automated rinsing can be tailored to suit the analysts' priorities (e.g., productivity or accuracy) as it improves throughput and/or minimizes sample-to-sample contamination.

Calibration

Calibration standards were prepared using Agilent single element standard calibration solutions. The calibration standards were matrix matched using H₂SO₄ and H₂O₂, then further diluted with Milli-Q water to form a final matrix of 18% H₂SO₄ and 2% H₂O₂. The calibration range used for each element is shown in Table 3. Linear calibration curves were achieved for all elements, as shown by correlation coefficients >0.9996 (Table 3). Representative calibration curves are shown in Figure 2.

Table 3. Background correction and calibration information.

Element and Wavelength (nm)	Background Correction	Calibration Range (ppm)	Correlation Coefficient
As 188.980	Fitted	0.005–50	1.00000
Cd 226.502	FACT	0.005–50	0.99997
Cu 324.754	Fitted	0.005–50	1.00000
Fe 239.563	Fitted	0.005–50	1.00000
Mn 294.921	Fitted	0.005–50	1.00000
Si 251.611	Fitted	0.01–50	0.99968
Ti 336.122	Fitted	0.005–50	1.00000
Zn 202.548	Fitted	0.005–50	1.00000

Background correction

The ICP Expert software includes automated background correction techniques so that the variable background structures arising from different sample matrices can be easily corrected. Fitted background correction (FBC) was used for all analyte wavelengths in this study, apart from Cd 226.502. FACT was used for Cd 226.502 due to interference from the high concentration of Fe in the decorative gold dust spray sample (Figure 3).

FBC automatically models the background signal under the analyte peak, providing accurate correction of both simple and complex background structures. No method development or manual placement of background peak markers is required for FBC. Sometimes, however, intense interferences that cannot be corrected using FBC can be corrected using FACT (Figure 3). FACT allows the analyst to select a specific interferent and analyte, removing the interferent emission intensity and ensuring accurate data for the analyte.

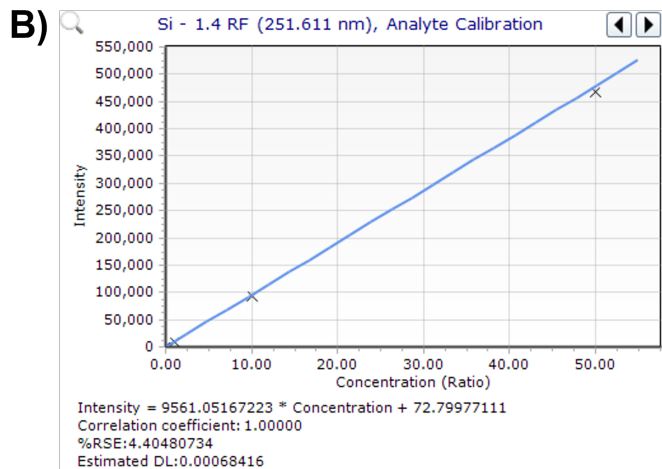
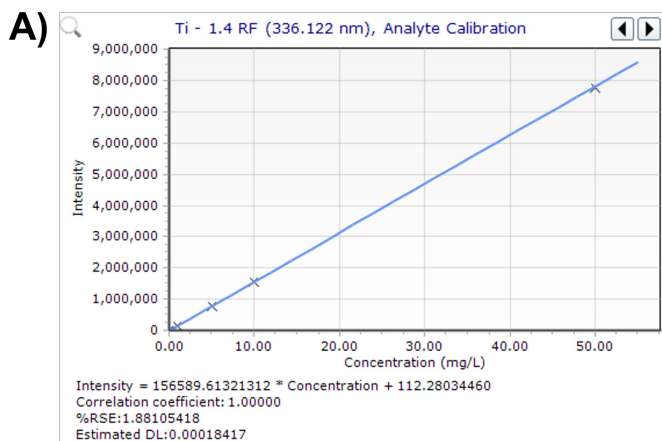


Figure 2. Representative calibration curves for Ti 336.122 nm (top) and Si 251.611 (bottom).

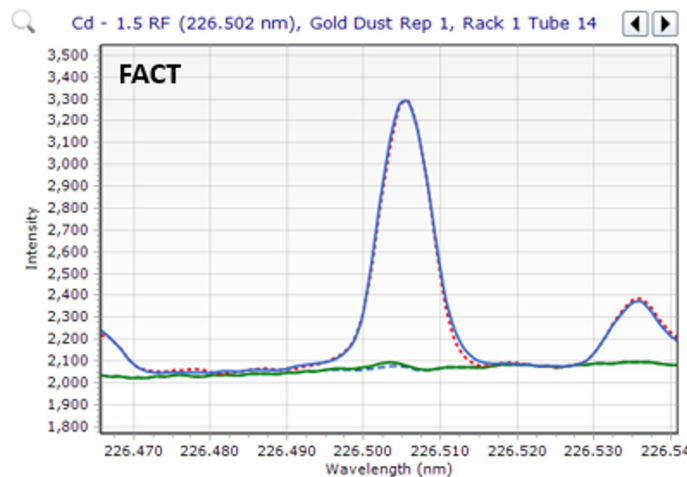
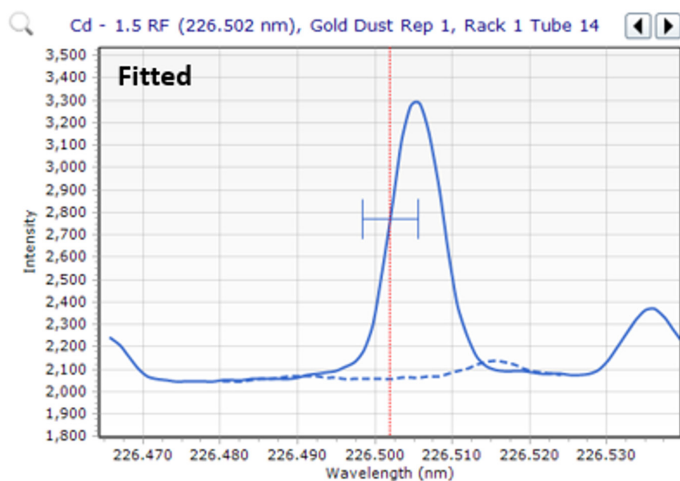


Figure 3. Comparison of FBC (left) and FACT (right) for background correction of the Fe interference on Cd 226.502. Key for FACT spectra: Raw spectral peak signal (blue solid line), blank (light blue dotted line), interferant peak (red dotted line), corrected analyte peak (green solid line).

Outlier conditional formatting (OCF)

The OCF feature within ICP Expert Pro alerts analysts of problematic results in real-time, so corrective action can be taken. This proactive approach saves time and costs by avoiding reruns of high numbers of samples. OCF uses color-based configurable rules to flag problematic results on the analysis screen. Results will be flagged if they are above a defined concentration range (overrange concentrations), have a high relative standard deviation (%RSD), are below the method detection limit, or if an internal standard (IS) exceeds an acceptable ratio.

OCF was used in this application to ensure that all results fell within the calibration range that was outlined in the ICP Expert software (0.005 to 50 ppm in most cases). An example of how OCF helped identify an IS delivery failure is shown in Figure 4. This type of real-time alert would enable the analyst to correct the problem before the end of a long analytical run, saving time and resources. In this case, the failure was caused by the consumption of all the sample solution. As the sample tubing was no longer taking up any sample solution, only the IS solution was being delivered to the plasma, approximately doubling the IS-to-sample ratio.

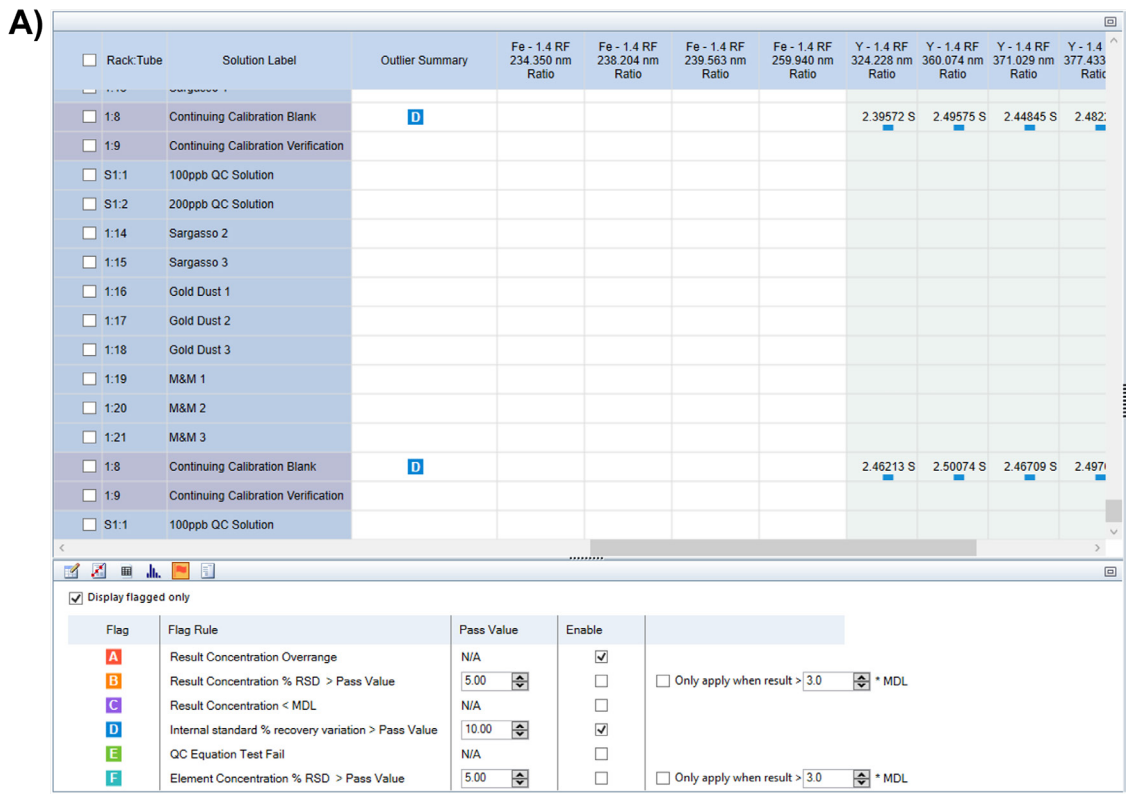


Figure 4A. Example of OCF triggering in response to an internal standard failure and an overrange result during an analytical run. The analyst can monitor internal standard recoveries, as well as change the calibration curve to fit the overrange data. In this example, four wavelengths of each element were selected.

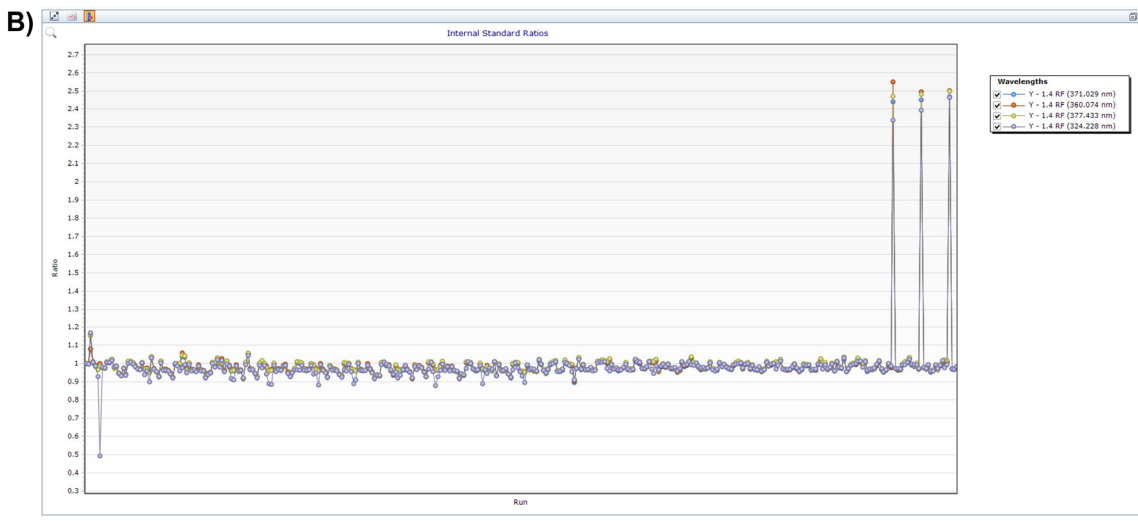


Figure 4B. Example of an internal standard ratio graph during an analytical run where internal standard failure was triggered by the OCF feature. The graph represents four different wavelengths of Y, all of which detected the internal standard failure, allowing the analyst to stop the run and refill the sample solutions to correct this problem.

Results and discussion

Detection limits

There are currently no set detection limit regulations regarding the concentration of TiO₂ (E171 food additive) in food products within the EU. To provide guidance on the detection limit requirements, the Chinese GB 5009.268-2016 specified limit of detection (LOD) and limit of quantification (LOQ) for Ti of 0.2 and 0.5 mg/kg, respectively, were used (Table 4).

The LODs and LOQs were calculated based on the analysis of 10 method blank solutions measured over six separate occasions on two separate instruments. The LODs were calculated by multiplying the standard deviation of the 10 blank replicates by three, while the LOQs were calculated by multiplying the standard deviation of the blank replicates by 10. As shown in Table 4, the 5800 ICP-OES quantitative LOD and LOQ values were below the detection limits specified in GB5009.268-2016.

Table 4. Comparison of Agilent 5800 ICP-OES LODs and LOQs with requirements specified in GB5009.268-2016. The LODs and LOQs were calculated based on sample preparation (0.50 g sample in 50 mL final volume).

Element and Wavelength (nm)	GB5009.268-2016 Analytical Limits (mg/kg)		5800 ICP-OES Quantitative Results (mg/kg)	
	LOD	LOQ	LOD	LOQ
As 188.980	NA	NA	0.37	1.26
Cd 226.502	NA	NA	0.02	0.08
Cr 205.560	NA	NA	0.03	0.12
Cu 324.754	0.2	0.5	0.05	0.19
Fe 239.563	1	3	0.05	0.18
Mn 294.921	0.1	0.3	0.02	0.07
Si 251.611	NA	NA	0.28	0.96
Ti 336.122	0.2	0.5	0.04	0.15
Zn 202.548	0.5	2	0.03	0.12

CRM analysis

Two CRMs were selected to verify the analytical method (sample digestion and ICP-OES data) used for the analysis of the various foods. Oyster tissue and seaweed (Sargasso) CRMs were selected for their varied matrix compositions and their inclusion of Ti. Oyster tissue is primarily composed of proteins (42.6 ± 1.3%) while Sargasso is primarily composed of carbohydrates (68%).

Each CRM was prepared in triplicate and each digest was then analyzed in triplicate. Recoveries for all reported analytes fell within ±10% of the certified value, as shown in Table 5. The excellent recoveries validate the suitability of this HF-free digestion method for the preparation of different food matrices and confirmed the accuracy of the 5800 ICP-OES method.

Table 5. Oyster tissue and Sargasso CRM data, n=3.

Element and Wavelength (nm)	Certified Conc (mg/kg)	Measured Conc (mg/kg)	Recovery (%)
NIST 1566b Oyster Tissue			
As 188.980	7.65	7.72	100
Cd 226.502	2.48	2.44	98
Cr 205.560	0.05*	0.05	104
Cu 324.754	71.6	71.0	99
Fe 239.563	205.8	207.9	101
Mn 294.921	18.5	19.1	103
Si 251.611	0.1*	0.104	104
Ti 336.122	12.2	12.0	98
Zn 202.548	1424	1393.1	97
NIES No. 9 Sargasso			
As 188.980	115	108.2	94
Cd 226.502	0.15	0.153	102
Cr 205.560	0.2	0.19	99
Cu 324.754	4.9	4.5	92
Fe 239.563	187	197	105
Mn 294.921	21.2	23.2	109
Si 251.611	0.1*	0.104	104
Ti 336.122	9	8.8	97
Zn 202.548	15.6	14.4	92

*Represents values that were spiked in-lieu of a certified value being provided.

Quantitative data

The quantitative data for the four food sample digests obtained using the 5800 showed that Ti was present in all samples (Table 6). The composition of the samples, which primarily consisted of carbohydrate, dairy/fat, and protein, represented a diverse range of matrices that can be digested using the sample preparation method.

Table 6. Quantitative results of food samples analyzed using the Agilent 5800 ICP-OES, n=3. Data has been corrected for weight and dilution factor.

Element and Wavelength (nm)	Sample 1 Confectionary (mg/kg)	Sample 2 Decorative Spray (mg/kg)	Sample 3 Salad Cream (mg/kg)	Sample 4 Protein Bar (mg/kg)
As 188.980	0.37	0.50	<0.37 (LOD)	0.46
Cd 226.502	<0.03 (LOD)			
Cr 205.560	0.21	0.42	0.14	0.17
Cu 324.754	5.05	<0.05 (LOD)	0.25	3.33
Fe 239.563	56.3	24443	13.1	31.1
Mn 294.921	4.97	22.2	0.42	1.93
Si 251.611	12.8	86.6	6.4	8.6
Ti 336.122	938	756	699	103
Zn 202.548	13.0	4.6	3.6	19.6

Comparative ICP-OES and ICP-MS quantitative data

To further confirm the validity of the H₂SO₄-acid digest sample preparation procedure and 5800 ICP-OES method, comparative data was obtained by ICP-MS. Agilent supplied Eurofins with aliquots of the homogenized food samples. The Eurofins team then prepared the samples using an HF-acid digest procedure and analyzed the digests using an Agilent 7900 ICP-MS.

The quantitative results for the confectionary product and decorative spray obtained using the two methods are shown in Figure 5. The graphs show that good agreement was achieved for the measurement of Ti (and other elements) in the two products by ICP-OES and ICP-MS. The results confirm the suitability of the safer HF-free acid digest sample preparation method for ICP-OES for the analysis of Ti in foods.

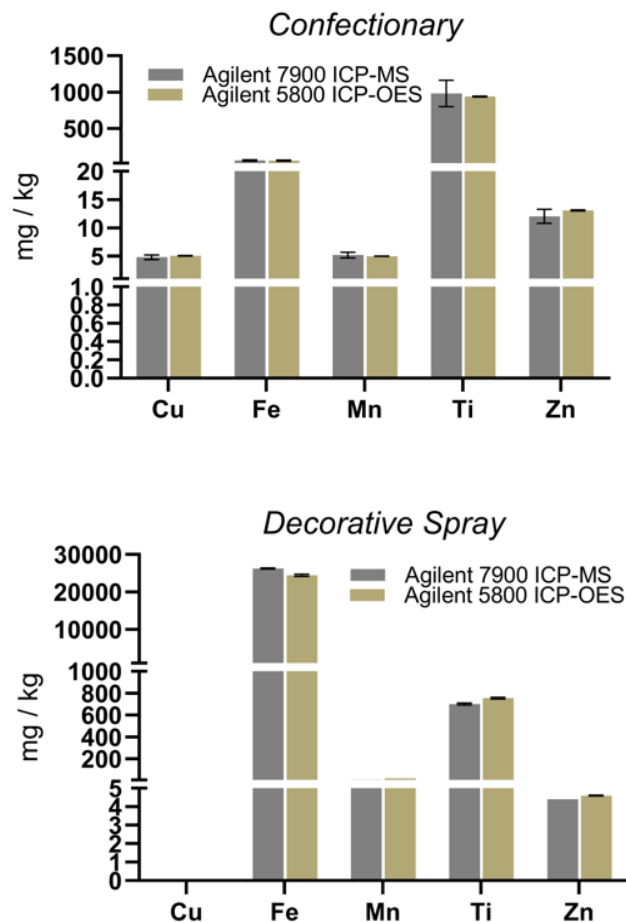


Figure 5. Comparison of Agilent 7900 ICP-MS + HF acid digest method (grey) and Agilent 5800 ICP-OES + H₂SO₄ acid digest method (yellow/green) results for the confectionary sample (top) and decorative spray sample (bottom).

Evaluation of matrix effects

To test for nonspectral interferences (matrix effects), Ti (IV) oxide, rutile >99.9% trace metals basis (Sigma-Aldrich, #224227-100G) was spiked at different concentrations into a method blank and into the Sargasso seaweed CRM. All results from the spiked recovery test show that both the digestion and analytical method are suitable for the complete recovery of TiO₂, confirming the absence of matrix effects (Table 7).

Table 7. Titanium dioxide (Ti (IV) oxide, rutile, >99.9% trace metals basis) spiked blank and spiked Sargasso CRM recovery values.

Spiked Concentration TiO ₂ (ppm)	Measured Total Titanium (ppm)	*TiO ₂ (ppm)	Total Recovery (%)
Blank Spike			
1	0.61	1.03	103
5	2.98	4.98	99
10	5.66	9.45	94
Sargasso CRM Spike			
1	0.65	1.08	103
5	3.10	5.17	102
10	5.95	9.93	98

*The concentration of titanium dioxide (TiO₂) was determined from the concentration of titanium (Ti). The ICP Expert software has a feature allowing analysts to add custom columns that apply stoichiometric conversion factors, such as automatically converting Ti concentration to TiO₂.

A fortified method blank was prepared by spiking the method blank with all elements from 0.02 to 0.5 ppm. To test for matrix effects arising from a food sample, a digested analytical salad cream sample was spiked with a similar concentration of each element that was already present in the sample. Table 8 shows that all elements were recoverable within 90–110%, providing further confirmation that the digestion and analytical method are suitable for the matrices under investigation.

Table 8. Spike recovery results, n=3.

Element, Wavelength (nm)	Fortified Method Blank		Fortified Analytical Portion				QC Criteria
	Spiked Concentration (ppm)	Recovery (%)	Unfortified (ppm)	Fortified (ppm)	Expected Spike (ppm)	Recovery (%)	
As 188.980	0.02	94	0.004	0.022	0.02	90	Pass
Cd 226.502	0.02	99	0.0001	0.021	0.02	105	Pass
Cr 205.560	0.02	99	0.002	0.023	0.02	105	Pass
Cu 324.754	0.02	90	0.003	0.023	0.02	100	Pass
Fe 239.563	0.5	96	0.156	0.660	0.5	101	Pass
Mn 294.921	0.02	98	0.005	0.026	0.02	105	Pass
Si 251.611	0.5	102	0.107	0.654	0.5	109	Pass
Ti 336.122	20	100	8.2	29.4	20	106	Pass
Zn 202.548	0.5	97	0.044	0.568	0.5	105	Pass

Long-term stability

To assess the stability of the 5800 ICP-OES, 300 solutions were measured over 10 hours without recalibration. The QC solution was measured directly after calibration and then after every 10 samples. Figure 6 shows the stability of the elements to be within ±10% over the run. The QC solution contained 100 ppb of each element in 18% H₂SO₄ and 2% H₂O₂. The %RSD was below 1.4% for all elements. The results demonstrate the excellent robustness, stability, and precision of the 5800 VDV ICP-OES for the routine QC analysis of foods and food additives such as TiO₂.

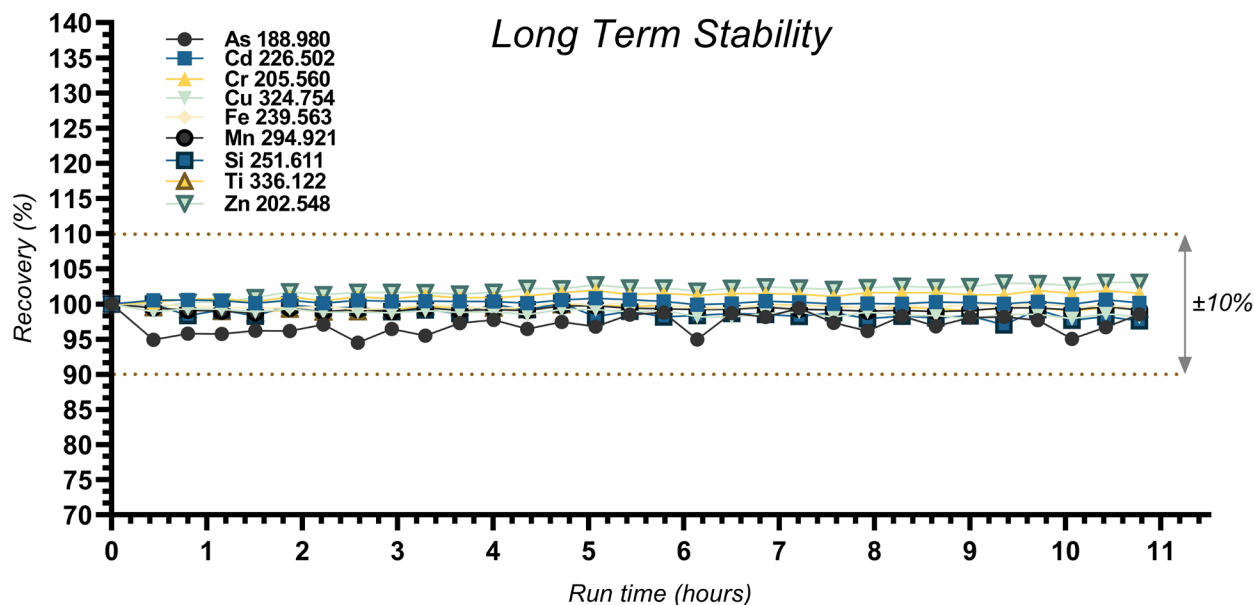


Figure 6. Recovery of all elements in a 100 ppb QC solution measured over 10 hours. The QC solution was measured every 10 samples and the samples ran in-between the QC solution were real digested samples that were analyzed in this study.

Conclusion

The Agilent 5800 VDV ICP-OES was used for the accurate analysis of multiple elements—including titanium—in a range of food products containing TiO_2 . The foods were prepared using a sulfuric acid-based microwave digestion procedure, avoiding the use of hydrofluoric acid.

The accuracy of the sample preparation and 5800 ICP-OES method was evaluated by analyzing two different CRMs, plus various spike recovery tests of the method blank, CRM, and sample. Recoveries were within $\pm 10\%$ in all cases, verifying the accuracy of the method for the measurement of multiple elements in foods, irrespective of the sample matrix. The suitability of the HF-free acid digest method for ICP-OES for the analysis of Ti in foods was confirmed by comparing data obtained for two samples prepared using HF and analyzed by ICP-MS. The instrument was stable over 10 hours of running complex food matrices, as shown by $\text{RSD}\% < 1.4\%$.

This work demonstrates the suitability of the 5800 VDV ICP-OES for the food manufacturing industry, specifically to identify and quantify the presence of TiO_2 in different foods. Also, following the EU ban of TiO_2 as a food additive (E171), the safer, HF-free ICP-OES method can be used to confirm the non-addition of E171 to foods.

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- Intelligent Rinse for ICP-OES, Agilent publication, [5991-8456EN](#)

Agilent part numbers

Description	Part Number
Easy-fit 1.8 mm semi-demountable torch for 5000 series VDV/SVDV ICP-OES	G8010-60236
Double-pass spray chamber, glass cyclonic design with ball joint socket and UniFit drain outlet	G8010-60256
SeaSpray concentric glass nebulizer for 5000 series ICP-OES	G8010-60255
Peristaltic pump tubing, white/white, 12/pk	3710034400
Peristaltic pump tubing, blue/blue, 12/pk	3710034600
Peristaltic pump tubing for internal standard, orange/white, 12/pk	3710046900
Y-piece connector for online addition of internal standard/ionization buffer	1610132400
Agilent 10,000 ppm single element stock solution for As, 500 mL	5190-8247
Agilent 10,000 ppm single element stock solution for Cd, 500 mL	5190-8328
Agilent 10,000 ppm single element stock solution for Cr, 500 mL	5190-8345
Agilent 10,000 ppm single element stock solution for Cu, 500 mL	5190-8349
Agilent 10,000 ppm single element stock solution for Fe, 500 mL	5190-8472
Agilent 10,000 ppm single element stock solution for Mn, 500 mL	5190-8484
Agilent 10,000 ppm single element stock solution for Si, 500 mL	5190-8522
Agilent 10,000 ppm single element stock solution for Ti, 500 mL	5190-8546
Agilent 10,000 ppm single element stock solution for Zn, 500 mL	5190-8558

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