

WHITEPAPER

X-ray Analysis

The Use of EDX Fluorescence
Spectrometry for Pharmaceutical
Elemental Impurity Analysis
Based on USP<735>



This whitepaper describes validation results using Cellulose, Talc and Mixture materials based the USP <735>. Performance depends on the material type. Please only use this whitepaper as a reference guide.

■ Introduction

New guidelines governing the analysis of elemental impurities in pharmaceuticals are being implemented. Toxic heavy metals and residual metal catalysts may exist in the raw materials of Active Pharmaceutical Ingredients (APIs) or be added during the manufacturing process. These impurities may be a risk to human health.

In December 2014, The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) issued the Q3D guideline, which limits 24 elements, including Cd, Pb, As, Hg, V, Co, Ni, Ir, Pt, Rh, Ru and Pd, in drug products and pharmaceutical ingredients. This guideline has reached the implementation stage. In May 2015, the United States Pharmacopeia (USP) established XRF methodology as a general chapter <735>, X-RAY FLUORESCENCE SPECTROMETRY. XRF spectrometry is a non-destructive analysis technique offering high sensitivity, precision, and accuracy without requiring chemical pretreatment.

This white paper demonstrates that X-ray Fluorescence (XRF) is capable of performing elemental impurity analysis of the above 12 elements in various pharmaceutical materials, such as Cellulose, Talc and a Mixture of Cellulose, Talc and TiO₂, by the calibration curve method using water solution standard samples, and verifying the qualification of USP <735>.

■ Permitted Daily Exposures and Concentration Limits

The ICH Q3D guideline defines the limit values as Permitted Daily Exposure (PDE) for oral, parenteral and inhalational drug products. Twenty-four elements are included in the risk assessment and are classified into four groups: Class 1 (Cd, Pb, As and Hg), Class 2A (V, Co and Ni), Class 2B (Ti, Au, Pd, Ir, Os, Rh, Ru, Se, Ag and Pt) and Class 3 (Li, Sb, Ba, Mo, Cu, Sn and Cr) (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, 2014).

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Classes 1 and 2A are very important elements and must be analyzed for regardless of whether they are added intentionally and throughout the production process. Class 2B elements are not required to be checked for the risk assessment if these elements are not intentionally added; however, catalyst elements such as Ir, Pt, Rh, Ru and Pd are commonly used in the process of producing APIs. Table 1 shows PDE limits of Class 1, 2A and 2B of the above five catalyst elements.

It is necessary to convert the maximum daily intake to concentration limits because the PDE units are µg/day. ICH Q3D guidelines advocate some conversion methods.

- 1) Option 1 is for common permitted concentration limits of elements across drug product components for drug products with daily intakes of not more than 10 grams.
- 2) Option 2a is for common permitted concentration limits across drug product components for a drug product with a specified daily intake.
- 3) Option 2b is for permitted concentration limits of elements in individual components of a product with a specified daily intake.
- 4) Option 3 is for permitted concentration limits from finished product analysis.

The most important thing is to change the concentration limits depending on the conversion method. Table 2 shows the oral concentration limits from PDE by options 1 and 2a for the assessment elements of Table 1. The maximum daily intake assumes 1.0 gram for option 2a.

Table 1: Permitted Daily Exposures for Elemental Impurities

Element	Class	Oral PDE µg/day	Parenteral PDE µg/day	Inhalation PDE µg/day
Cadmium, Cd	1	5	2	2
Lead, Pb	1	5	5	5
Arsenic, As	1	15	15	2
Mercury, Hg	1	30	3	1
Vanadium, V	2A	100	10	1
Cobalt, Co	2A	50	5	3
Nickel, Ni	2A	200	20	5
Iridium, Ir	2B	100	10	1
Platinum, Pt	2B	100	10	1
Rhodium, Rh	2B	100	10	1
Ruthenium, Ru	2B	100	10	1
Palladium, Pd	2B	100	10	1

Table 2: Oral Concentration Limits by Options

Element	Class	Concentration Limit by Option 1 (µg/g)	Concentration Limit by Option 2a (µg/g)*
Cadmium, Cd	1	0.5	5.0
Lead, Pb	1	0.5	5.0
Arsenic, As	1	1.5	15
Mercury, Hg	1	3.0	30
Vanadium, V	2A	10	100
Cobalt, Co	2A	5.0	50
Nickel, Ni	2A	20	200
Iridium, Ir	2B	10	100
Platinum, Pt	2B	10	100
Rhodium, Rh	2B	10	100
Ruthenium, Ru	2B	10	100
Palladium, Pd	2B	10	100

*Maximum daily intake is assumed as 1.0 gram.

■ X-ray Fluorescence (XRF) Spectrometry

The XRF technique does not require chemical pretreatment, and being a non-destructive analysis, can identify and determine the concentrations of elements in solid, powdered and liquid samples. XRF is capable of measuring a wide elemental range and at levels below part per million to percent concentrations.

There are two types of XRF spectroscopy techniques: Wavelength dispersive (WDX) and Energy dispersive (EDX). WDX is comprised of an X-ray tube, crystal monochromator and a counter detector. Multiple fluorescence X-rays generated from the sample are separated by a monochromator as individual fluorescence X-rays and measured by scanning the detector using a goniometer. WDX can perform analysis with high resolution and high accuracy; however, the system footprint is large and these systems have much higher power loading on the sample compared to EDX systems. The high-power X-ray source in WDX may cause browning and discoloring of the sample, especially for organic samples used in pharmaceutical drug materials.

EDX systems are comprised of an X-ray tube and semiconductor detector and can simultaneously capture many types of fluorescence X-rays generated from the sample using a multi-channel analyzer. EDX has lower resolution compared to WDX; however, EDX is a simultaneous technique capable of measuring all elements in the sample at the same time. EDX uses a lower power X-ray source, and will not significantly damage the sample. EDX systems are very convenient for users. In addition, EDX systems are smaller than WDX, they do not require any external utilities, such as chillers or gases, they use a 100V power supply, and they have no moving parts when measuring.

Quantitation is conducted using external calibration standards containing varying concentrations of elements. XRF calibrations have the advantage of being suitable for long periods of time before requiring recalibration, in contrast to other elemental analysis methods, such as Inductively Coupled Plasma (ICP) or Atomic Absorption Spectroscopy (AAS).

As the bulk of pharmaceutical materials are organic in nature, the focus on this white paper is to emphasize the compatibility, usefulness and benefit of EDX. This technique has the best potential to non-destructively measure the aforementioned twelve elements in drug materials without the need for chemical pretreatment.

Figures 1, 2 and 3 show the system configuration, a picture of an EDX system, and a typical spectrum of a cellulose sample containing some risk assessment elements.

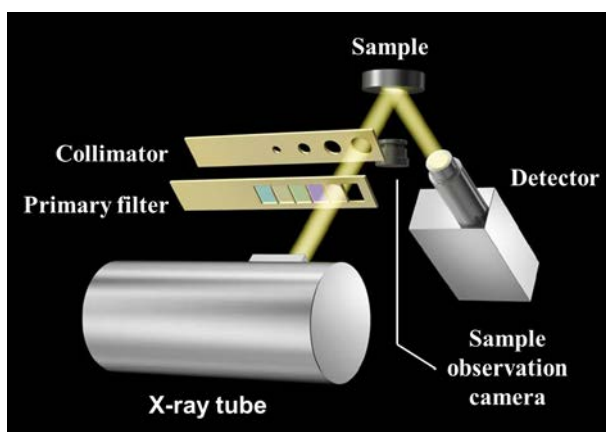


Figure 2: EDX-7000

Figure 1: Configuration of EDX system

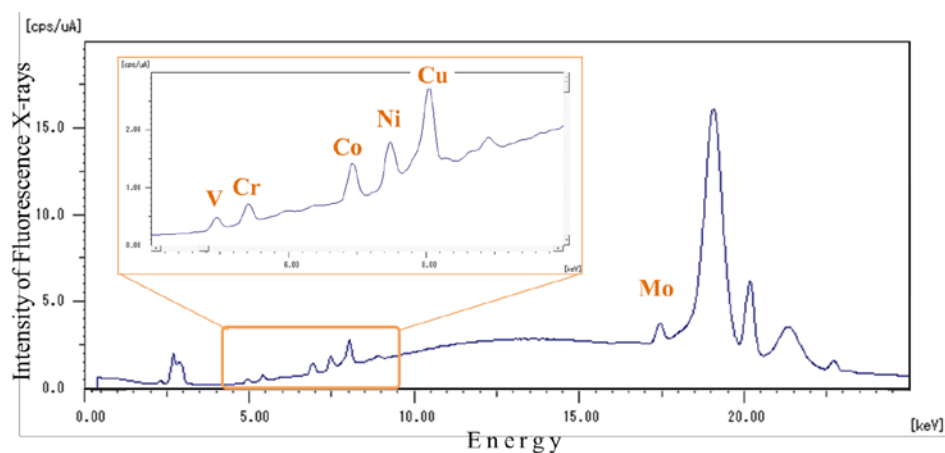


Figure 3: Typical EDX spectrum of a cellulose sample containing regulated elements

■ Impurity Control Method Proposal for Drug Materials

USP<233> specifies ICP-OES and ICP-MS techniques for elemental impurities analysis. ICP systems are more sensitive compared to XRF spectrometers; however, they require chemical preparation to liquefy the samples before measurement. This process is very complicated and labor intensive and can be influenced by human error. To reduce these complicated preparation procedures for ICP-OES and ICP-MS, the EDX technique can be utilized as a screening tool to determine if additional chemical analyses are required.

Furthermore, the XRF method may achieve similar performance for low-level chemical analysis if the measurement time is extended, as the detection limit improves with increasing time.

Figure 4 shows a proposal for a practical impurity control method for drug materials. This method consists of two techniques: screening analysis using EDX and verification using ICP-MS if EDX cannot detect the twelve risk assessment elements. This initial EDX screening method is effective for reducing analysis time and cost by minimizing the number of samples requiring precise analysis using ICP-MS.

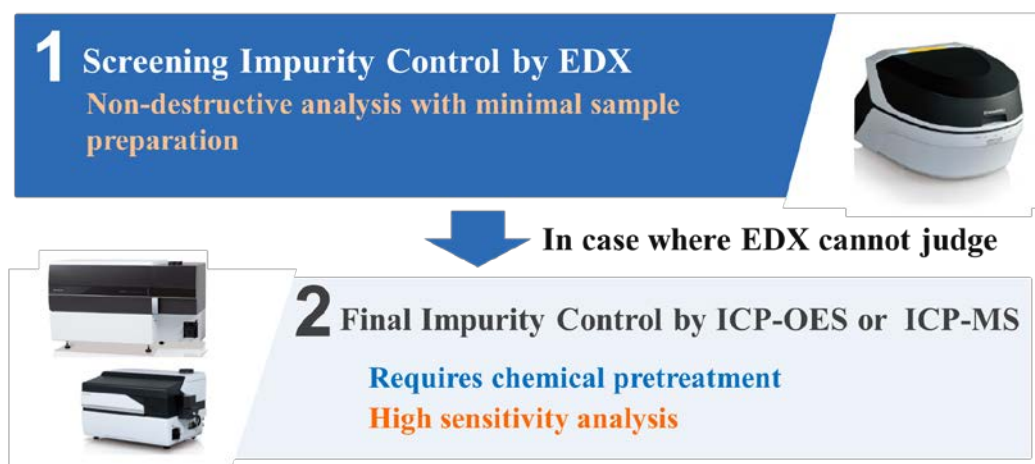


Figure 4: Proposal of practical impurity control method for drug materials

■ Sample Preparation

Solid, liquid and powder samples can be analyzed by XRF without any preparation. The only preparation required for XRF is reducing the sample to a size that fits in the sample cell or sample chamber. Basically, the larger the sample volume, the smaller the sampling errors.

Almost all solid samples can be placed into the sample chamber as is. Liquid samples are poured in a sample cell, with a supporting film at the bottom. Typical films are made from polypropylene of a few micrometers thickness. In general, polypropylene film has resistance to acids and bases. The majority of drug materials are in organic matrices and have relatively low X-ray absorption, allowing for the measurement of the elements that they contain. To ensure that the X-ray intensities are determined only by the composition and independent from the absolute volume, it is recommended to fill the sample cell to at least 5 mm in depth. Powder samples are placed directly into the sample cell using the tapping method to remove voids in the sample. Coarse powders must be ground to a fine particle size. Non-homogenous samples should be ground by means of mortar and pestle or a grinding device, such as a mill.

Figure 5 shows the procedure for assembling and packing a sample cell.

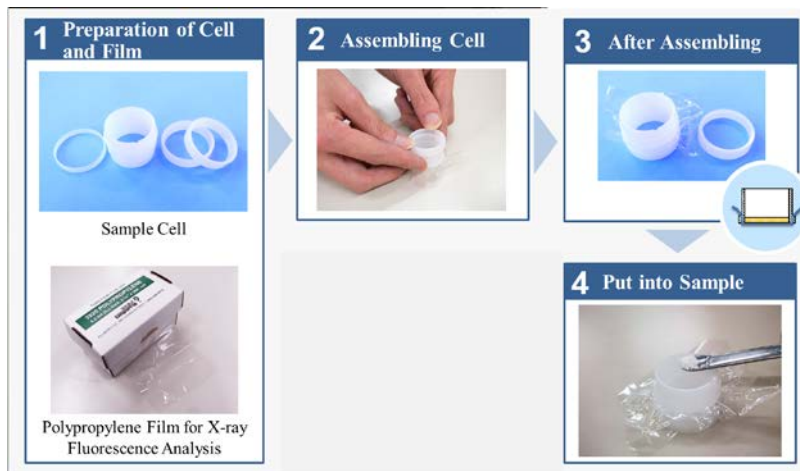


Figure 5: Procedure for assembling and filling a sample cell

■ Correction Method

XRF calibration is done by determining, for each element, the relationship between measured intensities and concentration using a number of standard samples. In general, the measured intensity is corrected by some spectral deconvolution method because the fluorescence X-ray intensity is affected by sample matrices, sample volume and sometimes interference with other peaks. These effects are well understood in XRF, and several methods have been developed to compensate for them, including:

Internal Correction Method

The internal correction method is used for the sample matrix and sample volume correction. This method uses fluorescence X-ray intensity and scattering X-ray intensity, such as Compton scattering or continuous scattered X-rays, and is used as the ratio of fluorescence X-ray intensity and scattered X-ray intensity.

Overlap Correction Method

The overlap correction method is used for the interference correction. This method subtracts the interference peak intensity from the measured overlap peak. The interference peak intensity can be evaluated by measuring other fluorescence X-ray lines for the interfering element because the intensity ratio of each line can be determined by measuring a single element sample.

In this risk assessment case, Pb and As have overlapping lines: $PbL\alpha$ and $AsK\alpha$. It is impossible to differentiate these two lines using only $PbL\alpha$ and $AsK\alpha$ because the energy of these two lines is very close and occurs in the same position. In this case, the different line, for example $PbL\beta_1$, is used to estimate the $PbL\alpha$ intensity in the total intensity of $PbL\alpha$ and $AsK\alpha$.

Figure 6 shows the cadmium intensities with different sample volumes and Table 3 shows the analytical results with and without correction.

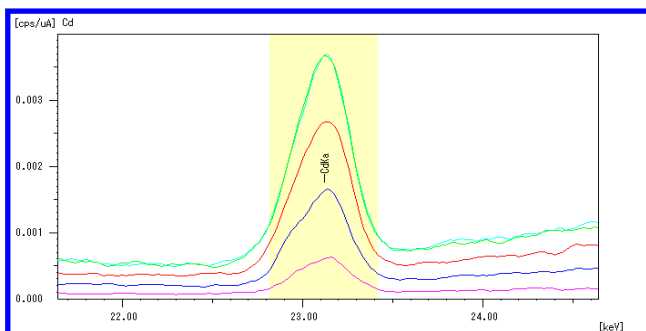


Figure 6: Overlaid cadmium spectra for several different sample volumes

Table 3: Analytical results with different sample volumes, with and without correction
Unit: $\mu\text{g/g}$, [] means deviation

Element	Sample	10 $\mu\text{g/g}$ Spiked Cellulose Powder		
	Sample Volume	2.0 g	1.0 g	0.5 g
⁴⁸ Cd	Without Correction	10.3 [0.9]	7.0 [0.8]	5.1 [0.6]
	With Correction	9.9 [0.9]	10.5 [1.1]	10.5 [1.3]
⁸² Pb	Without Correction	10.5 [0.5]	8.9 [0.4]	6.2 [0.2]
	With Correction	10.0 [0.5]	9.3 [0.5]	9.8 [0.4]
⁴⁵ Rh	Without Correction	11.6 [0.8]	8.9 [0.7]	6.9 [0.6]
	With Correction	10.7 [0.7]	10.4 [0.8]	10.7 [1.0]

■ Performance Qualification of USP <735>

USP <735> was enacted in May, 2015. The contents consist of Operational Qualification (OQ) and Performance Qualification (PQ). The purpose of OQ is to verify that the system operates within target tolerances using appropriate samples with known spectral properties. OQ checks key operating parameters, such as peak position, detector resolution and count rate, using specific metal samples. The purpose of PQ is to determine that the instrument is capable of meeting the user's requirements for all critical-to-quality measurements. There are some validation and verification items as follows:

1) Linearity

Analysts should demonstrate a linear relationship between the analyte concentration and corrected X-ray fluorescence response by preparing no fewer than five standards at concentrations that encompass the anticipated concentration of the test sample. The standard curve then should be evaluated using appropriate statistical methods such as least squares regression. The correlation coefficient (R), y-intercept, and slope of the regression line must be determined.

Acceptance Criteria: R is not less than 0.99.

2) Accuracy / Specificity

Analysts can determine accuracy by conducting recovery studies using the appropriate matrix spiked with known concentrations of elements. It also is an acceptable practice to compare assay results obtained using the X-ray fluorescence method under validation to those from an established analytical method.

Acceptance Criteria: 70.0% to 150.0% recovery

3) Repeatability

Analysts should measure the concentrations of three replicates of three separate samples.

Acceptance Criteria: The relative standard deviation is not more than 20.0%

4) Intermediate Precision

Analysts should establish the effect of random events on the method's analytical precision. Typical variables include performing the analysis on different days, using different instrumentation, or having two or more analysts perform the method. As a minimum, any combination of at least two of these factors totaling six experiments will provide an estimation of intermediate precision.

Acceptance Criteria: The relative standard deviation is not more than 25.0%.

5) Range

Range is the interval between the upper and lower concentration of an analyte in the sample for which it has been demonstrated by meeting the accuracy requirement.

100%-centered Acceptance Criteria: The range is 80% to 120%.

Non-centered Acceptance Criteria: 10% below the lower limit of specification to 10% above the upper limit of the specification.

6) Quantitation Limit

The limit of quantitation (LOQ) can be estimated by calculating the standard deviation of not less than six replicate measurements of a blank and multiplying by ten.

Acceptance Criteria: The analytical procedure should be capable of determining the analyte precisely and accurately at a level equivalent to 50% of the specification.

7) Robustness

The reliability of an analytical measurement should be demonstrated by deliberate changes to experimental parameters.

Acceptance Criteria: The measurement of a standard or sample response following a change in experimental parameters should differ from the same standard measured using established parameters by not more than +/- 20%.

■ Experimental

Energy dispersive X-ray fluorescence spectroscopy measurements were carried out using the Shimadzu EDX-7000 benchtop spectrometer (Figure 2). The spectrometer is equipped with an air-cooled X-ray tube (1000 μ A, 50kV, 50W) and an electronically cooled silicon-drift detector. The calibration samples and unknown samples were measured using a counting time of 1800sec. for each measurement channel and an X-ray beam size of 10mm diameter.

The calibration samples were prepared from combining two aqueous stock solutions of Cd, Pb, As, Hg, V, Co and Ni in Classes 1 and 2A, and for Ir, Pt, Ru, Rh and Pd in Class 2B. These standard solutions were prepared at more than five different concentrations including a blank by dilution with reagent water. The validation and verification samples were prepared from cellulose, talc, and a mixture of cellulose, talc and titanium oxide powders spiked at three different concentrations with the aforementioned elements using standard solutions for Atomic Absorption Spectroscopy. The standard solutions were added to the above three types of blank powder samples and mixed in an agate mortar to prevent adhesion to the walls. The prepared samples are summarized in Table 4.

Table 4: Prepared sample information

a) Standard Samples

Sample	Standard Sample for Class 1 and 2A Concentration (μ g/g)							Sample	Standard Sample for Class 2B Concentration (μ g/g)				
	Cd	Pb	As	Hg	V	Co	Ni		Ir	Pt	Ru	Rh	Pd
1-1	0	0	0	0	0	0	0	2-1	0	0	0	0	0
1-2	0.5	0.5	1.5	3	10	5	20	2-4	10	10	10	10	10
1-3	1	1	3	6	20	10	40	2-5	20	20	20	20	20
1-4	2.5	2.5	7.5	15	50	25	100	2-6	50	50	50	50	50
1-5	5	5	15	30	100	50	200	2-7	100	100	100	100	100

b) Validation and Verification Samples

Sample	Sample for Class 1 and 2A Concentration (μ g/g)							Sample	Sample for Class 2B Concentration (μ g/g)				
	Cd	Pb	As	Hg	V	Co	Ni		Ir	Pt	Ru	Rh	Pd
Low 1	1.3	1.3	4	9	27	13	45	Low 2	27	27	27	27	27
Middle 1	3	3	9	20	60	30	100	Middle 1	60	60	60	60	60
High 1	5.5	5.5	17	33	110	55	110	High 2	110	110	110	110	110

The 8.0mL solution sample and 2.0 g powder samples were placed directly into a sample cell (32mm dia.) with a 5µm polypropylene support film. The powder samples were prepared using a tapping method to settle them in the cell in an effort to reduce voids in the sample packing. 1.0 g and 0.5 g of powder samples were also placed directly into a sample cell using the tapping method with a 5µm polypropylene support film for robustness testing. All concentrations of calibration samples were used to generate a calibration curve for each of the above elements.

Continuous scattered X-rays at each specific energy range for the above elements were used as an internal standard to correct for sample matrix and volume effects. The quantitation of the impurity elements was calculated by the ratio of fluorescent X-rays for each element with continuous scattered X-rays. The measurement conditions are summarized in Table 5.

Table 5: Measurement conditions

a) For Class 1 and 2A

Element	Voltage (kV)	Automatic Current Adjustment	Measurement Time (sec.)	Filter	Corrected Scattering Position	Element Line
Cd	50	Yes	1800	#1	High	CdK α
Pb				#4	Middle #2	PbL β 1
As						
Hg				Middle #1	HgL α	
Co						Middle #1
Ni	Middle #1	NiK α				
V			#2	Low	VK α	

b) For Class 2B

Element	Voltage (kV)	Automatic Current Adjustment	Measurement Time (sec.)	Filter	Corrected Scattering Position	Element Line
Ru	50	Yes	1800	#1	High	RuK α
Rh						RhK α
Pd				PdK α		
Ir	#4	Middle #2	IrL α			
Pt				PtL α		

■ Results

Linearity

Table 6 shows the linearity results. The correlation coefficient of all target elements for the calibration curve meets the acceptance criteria. Overlap correction is used for As because the energies of AsK α (10.53keV) and PbL α (10.55keV) are very close. The calibration curves for Cd, Pb, As, Hg, V, Co, Ni, Ir, Pt, Ru, Rh and Pd are shown in Figure 7, and overlaid spectra of standard samples for each element are shown in Figure 8.

Table 6: Correlation Coefficient Results

a) For Class 1 and 2A

Element	Cd	Pb	As	Hg	Co	V	Ni
Correlation Coefficient R)	0.9941	0.9975	0.9998	0.9999	0.9999	0.9999	0.9999

b) For Class 2B

Element	Ir	Pt	Ru	Rh	Pd
Correlation Coefficient R)	0.9997	0.9998	0.9999	0.9999	0.9999

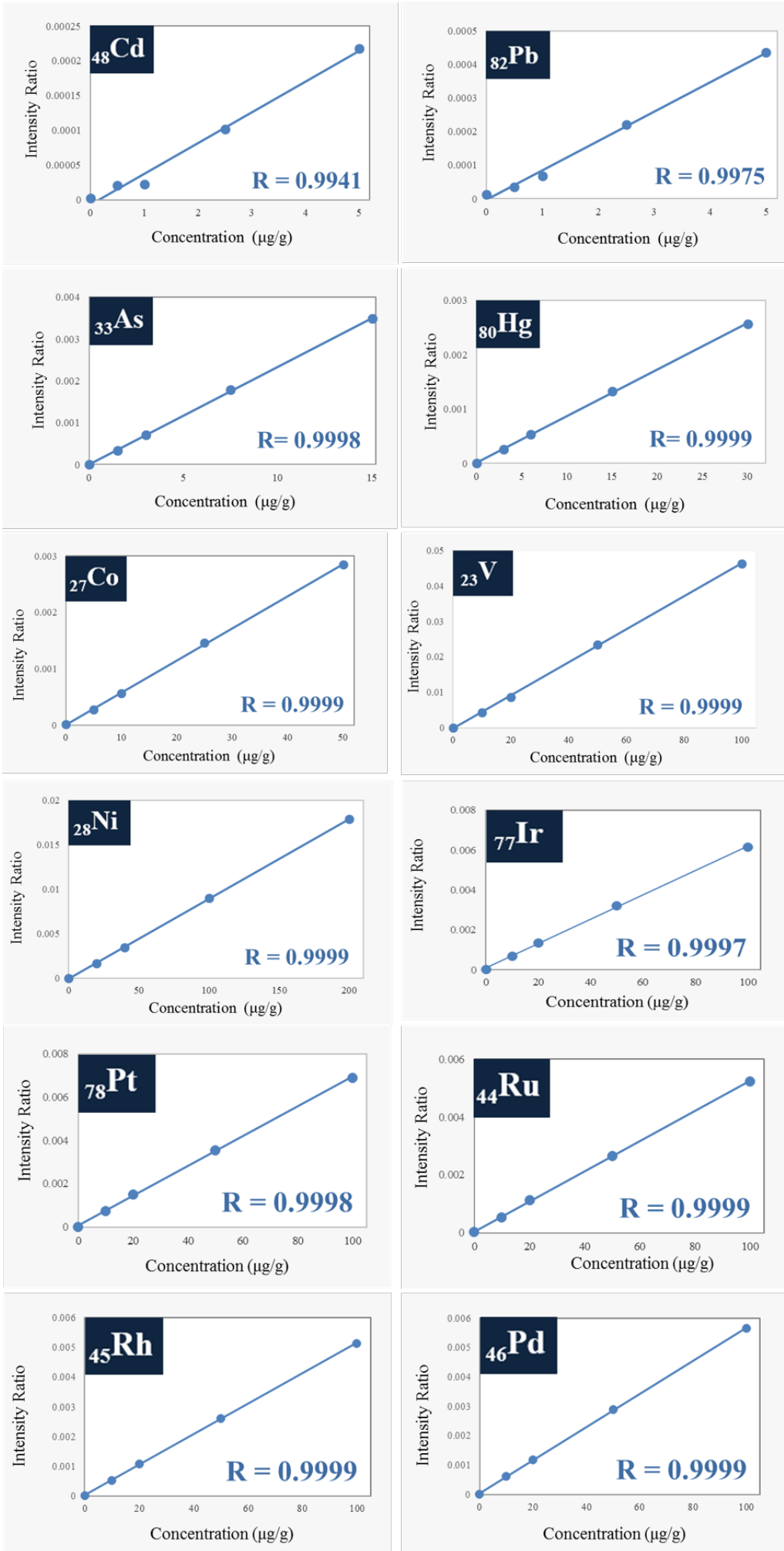


Figure 7: Linearity of Calibration Curves for Twelve Elements

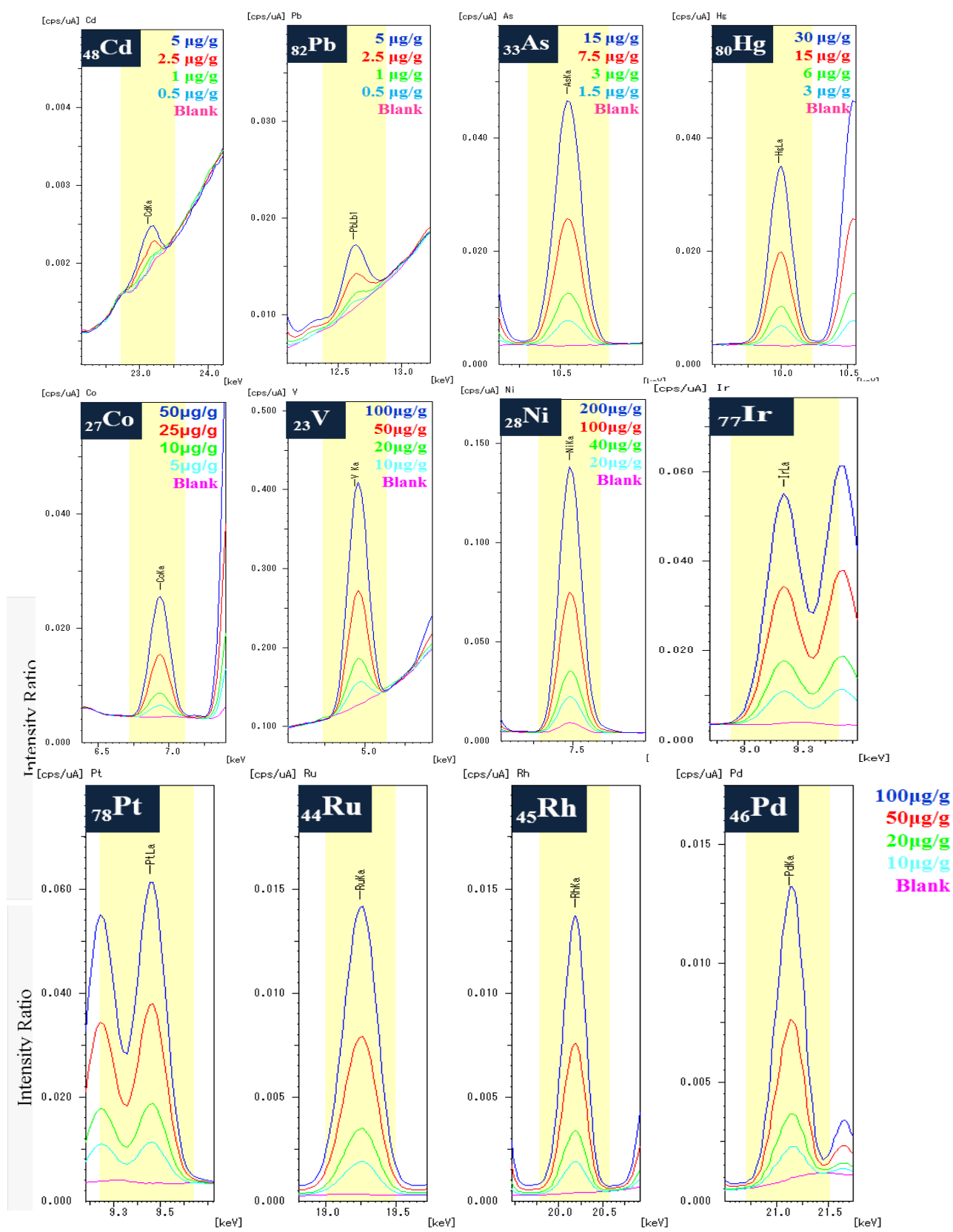


Figure 8: Overlaid Spectra of Standard Samples for the Twelve Elements

Accuracy / Specificity / Range

Table 7 shows the Accuracy / Specificity / Range results. In this white paper, the powder samples were quantitated by calibration using standard water solution sample(s). For the range, the concentration of 100%-centered range (80% to 120%) defined the concentration limit at a maximum daily intake of 1.0 g from PDE; thus, the "High" sample is within the 100%-centered range, and accuracy should be within 70.0% to 150.0%.

The "Middle" samples are in the non-centered concentration range, and accuracy was interpreted such that it should be within 63.0% to 165%. The mixture sample cannot validate / verify the V due to overlapping with TiK β derived from the component of titanium oxide in the mixture sample.

For the Talc sample, the elements at the low energy region less than 10 eV, such Hg, Ni, and V, have a low recovery rate because they are influenced by the matrix effect of other major components such as Si or Mg. However, the Accuracy / Specificity / Range of all target elements and matrices meet the acceptance criteria.

Figure 9 shows the overlaid Middle samples spectra of cellulose, talc and mixture.

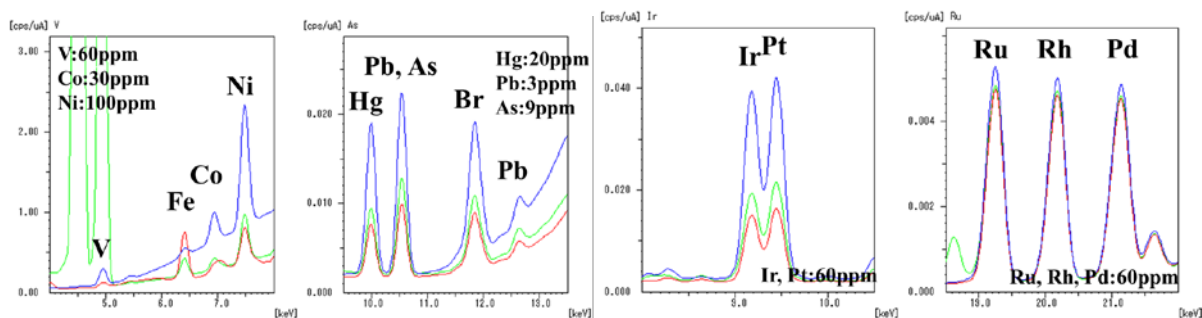


Figure 9: Overlaid Middle samples spectra of cellulose (blue), talc (red) and mixture (green)

Table 7: Accuracy / Specificity / Range Results

a) For Class 1 and 2A

Element Sample	Quantitative value ($\mu\text{g/g}$) (Difference from the target concentration = Accuracy (%))						
	Cd	Pb	As	Hg	Co	V	Ni
Middle 1	3	3	9	20	30	60	100
High 1	5.5	5.5	17	33	55	110	220
Cellulose Middle 1	3.01 (100.2)	2.4 (79.6)	8.6 (95.2)	18.2 (91.1)	32.1 (107.0)	66.1 (110.1)	102.7 (102.7)
Cellulose High 1	5.9 (107.8)	5.2 (94.7)	16.6 (97.4)	32.0 (97.0)	63.2 (115.0)	124.6 (113.3)	238.8 (108.5)
Talc Middle 1	3.4 (111.8)	3.1 (105.9)	7.1 (78.4)	14.2 (70.8)	25.9 (86.4)	43.1 (71.8)	69.4 (69.4)
Talc High 1	6.2 (113.1)	6.1 (110.9)	13.6 (80.0)	23.9 (72.4)	47.4 (86.1)	77.4 (70.3)	157.8 (71.7)
Mixture Middle 1	3.0 (101.5)	3.7 (122.3)	7.5 (82.8)	14.8 (74.1)	25.2 (84.0)	— *	77.6 (77.6)
Mixture High 1	5.8 (105.7)	6.4 (117.0)	15.0 (88.1)	27.0 (81.8)	51.0 (92.7)	— *	184.0 (83.6)

*Cannot validate / verify the V due to overlapping with TiK β .

b) For Class 2B

Element Sample	Quantitative value (µg/g) (Difference from the target concentration = Accuracy (%))				
	Ir	Pt	Ru	Rh	Pd
Middle 2	60	60	60	60	60
High 2	110	110	110	110	110
Cellulose Middle 2	62.5 (104.1)	60.4 (100.7)	62.0 (103.4)	60.9 (101.6)	58.5 (97.5)
Cellulose High 2	115.9 (105.3)	112.0 (101.8)	113.2 (102.9)	111.2 (101.1)	106.1 (96.5)
Talc Middle 2	46.7 (77.8)	46.6 (77.7)	60.6 (101.0)	61.0 (101.6)	59.1 (98.4)
Talc High 2	86.7 (78.8)	86.1 (78.3)	109.4 (99.4)	109.6 (99.6)	109.1 (99.2)
Mixture Middle 2	57.0 (95.0)	57.6 (96.0)	60.8 (101.3)	60.8 (101.4)	58.8 (98.0)
Mixture High 2	102.0 (92.7)	102.4 (93.1)	107.6 (97.9)	107.6 (97.8)	106.2 (96.5)

Repeatability

Table 8 shows the Repeatability results. In this Repeatability test, only the "Middle" samples were checked. The relative standard deviation was calculated from a total of nine data points for each material by measuring three replicates of three separate samples. All target elements and matrices meet the acceptance criteria.

Table 8: Result of Repeatability

a) For Class 1 and 2A

Element Standard	Average Quantitation (µg/g) (Relative Standard Deviation (%))						
	Cd	Pb	As	Hg	Co	V	Ni
Middle 1	3	3	9	20	30	60	100
Cellulose	2.9 (7.2)	2.5 (4.0)	8.3 (0.8)	18.4 (0.2)	31.3 (1.1)	65.7 (1.0)	102 (0.7)
Talc	3.1 (5.5)	3.0 (3.2)	7.1 (1.4)	14.4 (1.7)	26.1 (0.4)	42.5 (1.8)	69.9 (0.4)
Mixture	2.8 (12.4)	3.7 (3.3)	7.3 (1.0)	14.8 (0.8)	25.4 (1.4)	—	77.3 (0.1)

b) For Class 2B

Element Standard	Average Quantitation (µg/g) (Relative Standard Deviation (%))				
	Ir	Pt	Ru	Rh	Pd
Middle 2	60	60	60	60	60
Cellulose	58.2 (0.5)	56.2 (0.7)	59.1 (0.6)	57.8 (0.5)	54.4 (0.4)
Talc	45.9 (1.0)	45.8 (0.6)	58.3 (0.6)	59.2 (1.1)	56.8 (0.7)
Mixture	52.7 (0.8)	53.5 (0.4)	56.4 (0.2)	56.6 (0.4)	55.2 (0.7)

Intermediate Precision

Table 9 shows the Intermediate Precision results. In this Intermediate Precision test, only the “Middle” samples were checked. The variable test checked different days (two days), different samples (three separated samples), different instrumentation (two EDX-7000 instruments) and different analysts (two analysts). It is not a problem to apply different combinations of the above events, such as using three analysts instead of two instruments, if it is difficult to utilize more than two instruments. The relative standard deviation was calculated from a total of twelve data points for each event. All target elements meet the acceptance criteria.

Table 9: Intermediate Precision Results

a) For Class 1 and 2A

Element			³³ As	⁸⁰ Hg	⁸² Pb	⁴⁸ Cd	²³ V	²⁶ Co	²⁷ Ni
Laboratory A	First Day	1 time	8.47	18.20	2.33	2.61	65.87	32.21	102.52
		2 times	8.60	18.18	2.35	2.80	65.47	31.82	102.59
		3 times	8.57	18.23	2.39	3.01	66.08	32.10	102.70
	Second Day	1 time	8.52	18.13	2.38	2.75	66.63	32.02	102.88
		2 times	8.39	18.04	2.50	2.99	66.41	31.84	102.59
		3 times	8.48	18.06	2.39	2.88	65.49	31.58	102.79
Laboratory B	First Day	1 time	8.37	17.63	2.41	2.78	67.70	33.25	105.84
		2 times	8.24	17.34	2.52	2.93	67.46	32.67	105.76
		3 times	8.43	17.52	2.35	2.95	67.22	32.96	105.52
	Second Day	1 time	8.50	17.48	2.24	2.83	66.69	33.15	105.45
		2 times	8.43	17.63	2.41	2.51	68.12	32.90	105.46
		3 times	8.39	17.42	2.39	2.39	67.75	32.70	105.64
Average			8.45	17.82	2.39	2.78	66.74	32.43	104.14
Standard Deviation			0.097	0.345	0.074	0.194	0.908	0.572	1.538
CV (%)			1.1	1.9	3.1	7.0	1.4	1.8	1.5

b) For Class 2B

Element			⁷⁷ Ir	⁷⁸ Pt	⁴⁴ Ru	⁴⁵ Rh	⁴⁶ Pd
Laboratory A	First Day	1 time	62.51	60.35	61.80	60.96	58.17
		2 times	62.43	60.37	62.09	61.08	58.39
		3 times	62.63	60.46	61.96	60.75	58.58
	Second Day	1 time	62.14	60.36	61.94	61.32	58.52
		2 times	62.36	60.07	61.81	61.31	58.48
		3 times	62.12	60.04	61.74	60.83	58.25
Laboratory B	First Day	1 time	62.36	60.27	60.51	60.49	57.69
		2 times	62.50	60.87	61.00	60.79	57.43
		3 times	62.45	60.60	59.21	60.25	57.43
	Second Day	1 time	62.69	60.69	61.01	60.55	57.61
		2 times	62.27	60.77	60.71	60.34	57.49
		3 times	62.26	60.69	58.93	60.34	57.32
Average			62.39	60.46	61.06	60.75	57.95
Standard Deviation			0.179	0.265	1.071	0.369	0.494
CV (%)			0.3	0.4	1.8	0.6	0.9

Quantitation Limit

The quantitation limit was calculated as the standard deviation of the intensity for ten measurements of a blank cellulose sample. The quantitation limits for all target elements are shown in Table 10 for measurement times of 1800 sec. with PDE and control limits in the top four rows. In this white paper, the control limit is defined as the PDE limit from ICH Q3D. The concentration limits shown are from conversion methods option 2a. The two types of control values are provided by USP <735> (50% of PDE) and the ICH Q3D guideline (30% of PDE). The quantitation limit can meet both control limits at 1.0 g maximum daily intake and the EDX system can perform screening measurements for impurity control in these cases.

Table 10: Quantitation Limit Results

Element Limit	Quantitation Limits (µg/g)											
	Cd	Pb	As	Hg	V	Co	Ni	Ir	Pt	Ru	Rh	Pd
PDE for Oral (µg/day)	5.0	5.0	15	30	100	50	200	100	100	100	100	100
Concentration Limit by Option 2a (µg/g)*	5.0	5.0	15	30	100	50	200	100	100	100	100	100
50% USP control (µg/g)	2.5	2.5	7.5	15	50	25	100	50	50	50	50	50
30% ICH control (µg/g)	1.5	1.5	4.5	9.0	30	15	60	30	30	30	30	30
Detection Limit @1800sec. (µg/g)	0.3	0.2	0.1	0.1	0.7	0.2	0.4	0.1	0.1	0.2	0.1	0.3
Quantitation Limit @1800sec. (µg/g)	0.9	0.5	0.3	0.4	2.2	0.7	1.4	0.5	0.3	0.6	0.4	1.1
Estimated Detection Limit @900sec (µg/g)	0.4	0.3	0.15	0.15	1.0	0.3	0.6	0.15	0.15	0.3	0.15	0.4
Estimated Quantitation Limit @900sec (µg/g)	1.3	0.7	0.4	0.6	3.1	1.0	2.0	0.7	0.4	0.8	0.6	1.6
Estimated Detection Limit @300sec (µg/g)	0.75	0.5	0.25	0.25	1.7	0.5	1.0	0.25	0.25	0.5	0.25	0.75
Estimated Quantitation Limit @300sec (µg/g)	2.2	1.3	0.75	1.0	5.4	1.7	3.5	1.3	0.75	1.5	1.0	2.7

*Maximum daily intake is assumed as 1.0 gram.

Robustness

USP <735> proscribes the reliability of an analytical measurement should be demonstrated by deliberate changes to experimental parameters. In this white paper, the experimental parameter was defined as sample volume. Table 11 shows the robustness results for three different sample volumes: 2.0 g, 1.0 g and 0.5 g. The quantitation of 1.0 g and 0.5 g compare to that of 2.0 g as the robustness test. All target elements meet the acceptance criteria.

Table 11: Robustness Results

a) Classes 1 and 2A

Element Sample	Quantitative value (µg/g) (Difference from the quantitative value between 2.0 g and others (%))						
	Cd	Pb	As	Hg	Co	V	Ni
Middle 1	3	3	9	20	30	60	100
Cellulose, 2.0g	2.8	2.5	8.4	18.2	31.7	65.3	102.2
Talc, 2.0g	3.1	3.0	6.8	10.9	24.6	41.3	64.9
Mixture, 2.0g	2.9	3.6	7.4	14.8	25.2	—*	77.3
Cellulose, 1.0g	3.0 (8.8)	2.2 (9.1)	8.4 (0.6)	17.8 (2.2)	32.1 (1.2)	67.6 (3.4)	102.1 (0.5)
Talc, 1.0g	3.4 (8.3)	2.9 (5.4)	6.8 (1.1)	10.5 (4.2)	24.8 (0.6)	41.4 (0.2)	64.9 (0.3)
Mixture, 1.0g	2.4 (16.8)	3.5 (5.0)	7.5 (0.6)	14.8 (0.2)	25.2 (0.1)	—*	77.8 (0.7)
Cellulose, 0.5g	2.9 (3.6)	2.3 (6.2)	7.9 (5.7)	17.4 (4.6)	32.4 (2.3)	74.5 (14.0)	99.0 (3.1)
Talc, 0.5g	3.5 (12.5)	2.5 (15.8)	6.7 (1.5)	10.0 (8.1)	24.7 (0.4)	42.3 (2.4)	66.7 (2.9)
Mixture, 0.5g	2.5 (11.9)	3.1 (14.6)	7.3 (1.6)	14.8 (0.1)	26.4 (4.7)	—*	79.3 (2.6)

*Cannot validate / verify the vanadium due to overlapping with TiKβ.

b) Class 1 and 2A

Element Sample	Quantitative value (µg/g) (Difference from the quantitative value between 2.0 g and others (%))				
	Ir	Pt	Ru	Rh	Pd
Middle 2	60	60	60	60	60
Cellulose, 2.0g	62.4	60.4	61.9	60.7	58.4
Talc, 2.0g	46.6	46.4	59.8	60.6	58.3
Mixture, 2.0g	56.4	57.0	60.1	60.4	58.9
Cellulose, 1.0g	63.0 (0.9)	60.9 (0.8)	64.1 (3.6)	62.7 (3.2)	58.3 (0.3)
Talc, 1.0g	46.7 (0.3)	46.4 (0.1)	63.4 (6.0)	62.9 (3.9)	59.2 (1.4)
Mixture, 1.0g	57.3 (1.6)	56.8 (0.3)	61.9 (3.0)	61.6 (1.9)	58.7 (0.3)
Cellulose, 0.5g	63.6 (1.8)	60.6 (0.2)	61.5 (0.6)	58.8 (1.5)	57.8 (1.0)
Talc, 0.5g	47.9 (2.8)	47.0 (1.4)	61.2 (2.3)	58.8 (2.9)	58.1 (0.3)
Mixture, 0.5g	57.7 (2.3)	56.9 (0.1)	58.8 (2.1)	57.6 (4.7)	57.0 (3.2)

■ Conclusion

The Shimadzu EDX-7000 meets USP<735> acceptance criteria for linearity, accuracy, specificity, range, repeatability, intermediate precision, quantitation limit and robustness. If the maximum daily intake is set at 1.0 gram, EDX can be applied to the screening and control of impurities for the most important risk assessment elements of Cd, Pb, As, Hg, V, Co, Ni, as well as for important catalyst elements of Ir, Pt, Ru, Rh and Pd in the pharmaceutical field. EDX reduces the need for complicated chemical analysis procedures requiring time-consuming chemical preparation processes.

■ Summary

1. It is becoming necessary to control elemental impurities for drugs according to the guidelines from ICH Q3D.
2. ICP-OES or ICP-MS are powerful tools for elemental impurity analysis; however, chemical preparation is always necessary. The XRF method can perform qualitative and quantitative elemental analysis with non-destructive and chemical-free preparation. This method can be used as a "Screening" tool to determine the need for verification testing using chemical analysis methodology.
3. In verification for USP <735>, a calibration curve generated from a standard aqueous solution, linearity, accuracy, specificity, repeatability, intermediate precision, quantitative limit and robustness using Cellulose, Talc and Mixture meet the acceptance criteria.

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