analytical techniques

Elemental analysis by WD-XRF: A simplified approach

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Using wavelength dispersive x-ray fluorescence (WD-XRF) to assess elemental impurities offers several advantages over other techniques. It eliminates the need to prepare a solution and is more suitable for use in a manufacturing setting. This article summarizes a study that illustrates the advantages of a direct method of solid analysis and discusses direct analysis of a cup of finished tablets without need for sample preparation.

New guidelines from the International Conference on Harmonization (ICH) call for the pharmaceutical industry to test its products for elemental impurities. The document, ICH Q3D "Elemental Impurities" [1] has been incorporated into an FDA Guidance [2] and General Chapters <232> and <233> from United States Pharmacopeia (USP) outline the impurity limits and suggest analytical procedures [3,4].

While these documents suggest methodologies and sample preparation procedures, they are not rigid because the variability of the materials and the range of elemental analytes require flexibility in conducting the tests. Even so, inductively coupled plasma (ICP) techniques have been the most widely used due to their high sensitivity.

Both ICP mass spectroscopy (ICP-MS) and ICP optical emission spectroscopy (ICP-OES) tend to be very sensitive (≤1 part per billion) for many elements, but they require digestion of the sample material into a solution [5-7]. For final product analyses of tablets and capsules, this would require materials to be ground and dissolved in very strong acids (in some cases highly toxic hydrofluoric acid) with the addition of agitation or heat. In many cases, a microwave digestion apparatus is required [5]. Certain hard-to-digest samples may not dissolve completely or the solutions that result may require dilution before undergoing final analysis. To prevent contamination of the materials, these preparatory steps must often be performed in a cleanroom. In addition, ICP techniques require skilled operators and must often be closely monitored during analyses. As a result, these techniques are not commonly implemented in a manufacturing setting to conduct final product quality assurance/quality control (QA/QC).

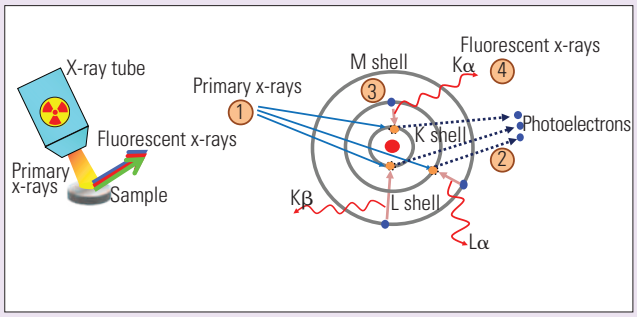
This article describes how wavelength dispersive x-ray fluorescence (WD-XRF) can be used to detect and quantify elemental impurities in compliance with ICH Q3D. It also discusses how WD-XRF—a direct technique that eliminates sample digestion—can analyze final products as unadulterated tablets in a nondestructive way. For simplicity, a cellulose matrix was used in the study described here. Different formulations, however, have shown similar results.

WD-XRF has been implemented in QA/QC operations in many industries, including steel and alloys, cement, plastics, glass, food, and oil production and refining, where it is accepted as a stable, reliable, and simple-to-operate technique. In basic terms, WD-XRF entails excitation of atoms in a solid or liquid sample in order to produce characteristic x-rays that are separated by wavelength dispersion and detected.

The diagram in Figure 1 illustrates how fluorescent x-rays are generated. Because secondary fluorescent x-rays are characteristic of the atoms contained in the sample, they make it possible to identify the sample's constituents. Figure 2 shows the technique's specificity for

FIGURE 1

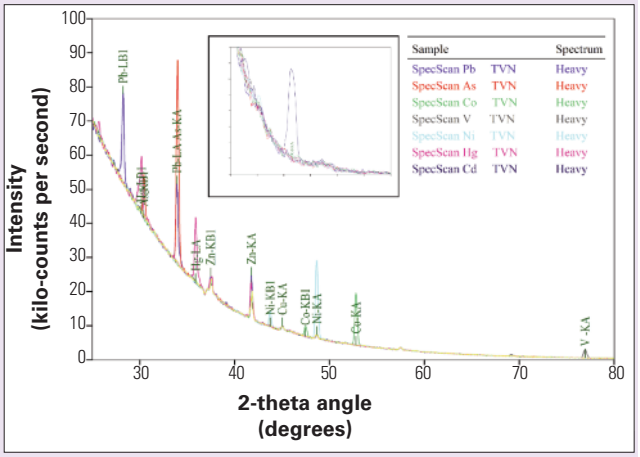
Generation of x-ray fluorescence



1. Primary x-rays strike inner-shell electrons.
2. An inner-shell electron is kicked out as a photoelectron.
3. An outer-shell electron transfers to fill the vacancy.
4. Fluorescent x-ray is emitted with equivalent energy difference.

FIGURE 2

Specificity: Qualitative heavy scans



the key elements of interest, as cited in ICH Q3D (Class 1 and 2A). The count rates of the detected x-rays are proportional to the concentration in the irradiated sample. Quantitative applications can be created by analyzing a set of known standards of analytes in the concentration ranges of interest. Typically this is done for a specific matrix type. Unlike ICP techniques, XRF methods do not need daily recalibration. In most cases, a monthly drift check using known samples reveals whether the calibration is within specification.

Traditionally, preparation of solid XRF samples involves grinding the material to a homogeneous particle size and compressing the powder into a solid pellet using a 10- to 30-ton press. The photo below shows a typical 5-gram pellet 40 millimeters (mm) in diameter. With no further preparation needed, the sample is simply placed into the designated cup and analyzed. While this two-step method is simpler than having to digest samples, it could be improved. In fact, we have developed and tested a method in which the analyst simply pours finished tablets into a cup and analyzes them. In the photos below, a set of oblong tablets and a whole pellet (for comparison) are ready for WD-XRF analysis. We describe the new method of analysis and its advantages below.

Typical 5-gram pellet 40 mm in diameter

A set of oblong tablets and a pellet ready for analysis. The ability to test finished tablets simplifies the analysis.

Methods

Equipment. All quantitative analyses were performed using a Rigaku WD-XRF Primus IV, which operates at 4 kilowatts at maximum power, with tube-above optics and a SuperTrace 30-micron-thick beryllium (Be) end-window rhodium (Rh) tube. The instrument is equipped with scintillation counter (SC) and flow-proportional counter (F-PC) detectors. The power setting (killivolt-milliamp) was optimized to achieve maximum excitation of elements that ICH Q3D classifies as Class 1 and Class 2A, i.e., lead (Pb), arsenic (As), cadmium (Cd), mercury (Hg), cobalt (Co), vanadium (V), and nickel (Ni). Peak and background positions were chosen for efficient counting times. Table 1 lists the instrument's operating parameters during the analysis of these elements.

Bulk powder mixing was done in a Retsch Mixer Mill E-MM-2000 (Premier Lab Supply, Port St. Lucie, FL).

TABLE 1

Representative parameters of instrument in operation

Parameter	Element						
	Hg-LA	Cd-KA	Pb-LB1	As-KA	Co-KA	V-KA	Ni-KA
Common conditions							
Emission line	KA, LA, LB1						
Tube target	Rh						
Atmosphere	Vacuum						
Meas. diameter (mm)	30						
Spin setting	On						
Primary beam filter	Out (exceptions for Cd- Ni400; Hg, Pb, As - Al125)						
Component type	Metals						
Balance	C6H1005 (cellulose)						
Element-specific conditions							
Power (kV-mA)	50-70	60-60	50-70	50-70	50-70	50-70	50-70
Slit	S2	S2	S2	S2	S2	S2	S2
Analyzing crystal	LiF(220)	LiF(220)	LiF(220)	LiF(220)	LiF(220)	LiF(220)	LiF(220)
Detector	SC	SC	SC	SC	SC	SC	SC
PHA setting	105-270	120-270	115-270	110-280	105-290	105-285	100-300
Peak angle (deg)	51.676	21.684	40.344	48.812	77.908	123.262	71.280
BG1 (deg)	51.240	21.320	40.040	48.240	77.380	122.240	72.140
BG2 (deg)	52.020	22.240	40.700	49.480	78.549	-	-
BG coefficient	-	-	-	-	-	-	-
Time - Peak (sec)	45	240	120	20	10	20	5
Time - BG (sec)	45/45	120/120	120/120	20/20	10/10	10	5

Pellets were prepared using the Atlas Automatic Hydraulic Press—40 Ton (set to 15 tons) equipped with a 40—mm die assembly (Premier Lab Supply). The tablet sets were prepared using a manual Model 3925 hydraulic press (Carver, Wabash, IN) equipped with both round and oblong TDP-0 tablet press punches and dies (Foshan KOS, Guangdong, China).

Materials. ICP standards for Pb, As, Cd, Hg, Co, V, and Ni were purchased from Fisher Scientific (Fair Lawn, NJ). All the ICP standards had a concentration of 1,000 ppm, ±1 ppm. Cellulose was purchased from SPEX SamplePrep (Metuchen, NJ), and polypropylene cups were purchased from Premier Lab Supply. A Milli-Q water purification system (Millipore, Bedford, MA) was used to produce the deionized water used in serial dilutions of the elemental "cocktails."

Standard preparation. Bulk powder material (cellulose matrix) was compressed into pellets and tablets so each could be analyzed and compared. Standard ICP solutions were used to prepare a cocktail of Class 1 and 2A elements. Serial dilutions were made accordingly. The cellulose powder was then spiked with the various diluted standards and dried to the desired dry-weight concentrations, which are listed in Table 2. Pellets were prepared for each concentration by placing 5 grams of powder in a 40-mm die assembly. Tablets were prepared by similar manual compression. Because each tablet contained approximately 250 milligrams of the cellulose matrix, approximately 20 tablets were used to constitute a 5-gram set for analysis. Tablet sets were simply poured into a polypropylene cup held in place by the cup holder. It was not necessary to use a film to keep the samples in place; the tube-above geometry of the spectrometer was sufficient. Sets of both round and oblong tablets were made to see how shape affected reproducibility and sensitivity.

TABLE 2

Standard concentration ranges of spiked powder

STDS	Cd	Pb	As	Hg	Co	V	Ni
LOQ	0.4	0.4	1.2	2.4	4	8	16
0.5J	0.5	0.5	1.5	3	5	10	20
1J	1	1	3	6	10	20	40
2J	2	2	6	12	20	40	80
ULOQ	5	5	10	20	30	50	100

Results and discussion

Correlation factors for all calibration curves ranged from 0.996 to greater than 0.9999 (Table 3). According to options 2 and 3 offered in ICH Q3D, it is permissible to set the detection limits according to final product daily intake. The limits of detection (LOD) listed in Table 3 were suitable for use with daily intakes equal to or greater than 5 grams of final product. The LOD for each element can be reduced by employing longer counting times, higher power, and a larger analysis area/cup size.

TABLE 3

Limit of quantitation, range, and linearity for pellets of each element of interest

Elements	LOD (ppm)	LOQ (ppm)	Target concentration** (ppm)	Range (ppm)	Linearity (R^2)
Cd	0.13	0.4	1	0.4 - 5	0.998188
Pb	0.20	0.4	1	0.4 - 5	0.999468
As	0.13	1.2	3	1.2 - 10	0.999973
Hg	0.28	2.4	6	2.4 - 20	0.996066
Co	0.71	4	10	4 - 30	0.999319
V	1.17	8	20	8 - 50	0.999755
Ni	0.59	16	40	16 - 100	0.999974

* LOD may be improved with a longer counting time.
** Target concentration is based on a 5-gram dose of finished product according to USP <232> for elemental impurities.

Tables 4 to 8 show typical validation results per USP <233>. The pellet method was initially used to demonstrate validation, and all aspects of the validation, including intermediate precision and ruggedness, were easily achieved. The goal of this study was to test whether voids and random or preferred orientation of the tablet sets affected the results relative to a pellet. We also sought to learn whether different tablet shapes affected repeatability and to what extent and whether calibrations for one tablet could be used to analyze another that had a significantly different shape.

TABLE 4

Detectability

Sample ID (average reported)	Cd (ppm)	Pb (ppm)	As (ppm)	Hg (ppm)	Co (ppm)	V (ppm)	Ni (ppm)
Standard solution 1	1.00 (9.0)	1.11 (5.4)	2.90 (0.7)	5.92 (4.1)	9.94 (2.6)	20.60 (4.1)	40.54 (1.6)
Spiked 1J	0.92 (1.1)	1.14 (5.1)	2.98 (1.2)	5.58 (5.7)	9.97 (2.7)	20.19 (1.1)	40.79 (1.5)
Spiked 0.8J	0.87 (2.9)	0.95 (8.2)	2.33 (2.7)	4.35 (4.8)	8.34 (4.1)	15.86 (2.5)	32.34 (0.8)
Acceptance criteria 1.	7.69	2.69	2.76	5.80	0.30	1.99	0.62
Acceptance criteria 2	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Notes: Three individual samples were prepared at the following concentrations containing the target elements: Spiked 0.8J, Spiked 1J, and Standard Solution 1J. Values reported are averages of three replicate measurements. Numbers in parentheses are percentage coefficient of variation.
Acceptance criteria:
1. The average value of the three replicate measurements of Spiked 1J is ±15% of the average value for the three replicate measurements of the Standard solution 1J.
2. The average value of the three replicate measurements of Spiked 0.8J < Standard solution 1.

TABLE 5

Precision and accuracy

Sample ID	Cd (ppm)	Pb (ppm)	As (ppm)	Hg (ppm)	Co (ppm)	V (ppm)	Ni (ppm)
Nominal concentration	1.00	1.00	3.00	6.00	10.00	20.00	40.00
Accuracy-1	1.07	1.10	2.92	5.49	9.96	21.20	39.88
Accuracy-2	0.94	1.12	2.91	5.37	9.67	19.94	40.45
Accuracy-3	0.98	1.05	2.85	5.30	9.67	19.94	40.41
Mean (ppm)	1.00	1.09	2.89	5.39	9.82	20.75	40.25
Precision (%RSD)	6.68	3.31	1.31	1.78	1.48	3.39	0.79
Accuracy (%)	100	109	96	90	98	104	101

Notes: Three individual samples were prepared at the 1J concentration containing the target elements.
Acceptance criteria for spike recovery: 70% to 150% for the mean of three replicate preparations.

Surprisingly, no significant loss in sensitivity or repeatability was observed for any of the elements studied (Table 9). Furthermore, when a calibration that was prepared using oblong tablets was in turn used to test round tablets, no difference or bias was observed in the

Table 6

Repeatability

Sample ID	Cd (ppm)	Pb (ppm)	As (ppm)	Hg (ppm)	Co (ppm)	V (ppm)	Ni (ppm)
Repeatability-1	0.91	1.06	3.03	5.33	10.65	21.26	40.73
Repeatability-2	1.08	1.16	2.98	6.05	10.59	20.62	40.90
Repeatability-3	1.15	1.17	2.95	5.53	10.12	20.19	40.41
Repeatability-4	1.02	1.17	2.94	5.47	10.28	20.25	40.22
Repeatability-5	0.97	1.11	2.93	5.22	9.98	20.94	40.72
Repeatability-6	0.97	1.04	2.98	5.74	10.06	21.03	40.55
Mean	1.02	1.12	2.97	5.56	10.28	20.72	40.59
Standard deviation	0.09	0.06	0.04	0.30	0.28	0.44	0.25
%RSD	8.52	5.17	1.23	5.40	2.74	2.10	0.61

Notes: Six individual samples were prepared at the 1J concentration containing the target elements. Acceptance criteria: %RSD ≤ 20% for each target element.

Table 7

Ruggedness

Sample ID	Cd (ppm)	Pb (ppm)	As (ppm)	Hg (ppm)	Co (ppm)	V (ppm)	Ni (ppm)
Repeatability-1	0.91	1.06	3.03	5.33	10.65	21.26	40.73
Repeatability-2	1.08	1.16	2.98	6.05	10.59	20.26	40.90
Repeatability-3	1.15	1.17	2.95	5.53	10.12	20.19	40.41
Repeatability-4	1.02	1.17	2.94	5.47	10.28	20.25	40.22
Repeatability-5	0.97	1.11	2.93	5.22	9.98	20.94	40.72
Repeatability-6	0.97	1.04	2.98	5.74	10.06	21.03	40.55
Ruggedness-1	1.03	1.02	2.98	3.94	10.33	21.49	40.33
Ruggedness-2	1.05	0.99	2.94	4.68	10.45	19.63	40.78
Ruggedness-3	0.93	0.93	3.04	4.48	9.96	19.90	40.71
Ruggedness-4	0.86	0.94	2.99	4.38	10.09	19.51	40.56
Ruggedness-5	1.06	1.01	2.99	4.46	9.92	20.14	40.26
Ruggedness-6	1.05	0.81	3.06	4.76	9.98	19.58	39.80
Mean	1.01	1.03	2.98	5.00	10.20	20.38	40.50
Standard deviation	0.08	0.11	0.04	0.64	0.25	0.68	0.31
%RSD	8.07	10.59	1.40	12.84	2.49	3.34	0.76

Table 8

Specificity

Sample ID	Cd (ppm)	Pb (ppm)	As (ppm)	Hg (ppm)	Co (ppm)	V (ppm)	Ni (ppm)
Hg pellet	BLOD	BLOD	BLOD	**112.09**	BLOD	BLOD	BLOD
Cd pellet	**93.51**	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Pb pellet	BLOD	**103.09**	BLOD	BLOD	BLOD	BLOD	BLOD
As pellet	BLOD	BLOD	**129.14**	BLOD	BLOD	BLOD	BLOD
Co pellet	BLOD	BLOD	BLOD	BLOD	**105.31**	BLOD	BLOD
V pellet	BLOD	BLOD	BLOD	BLOD	BLOD	**104.82**	BLOD
Ni pellet	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD	**106.68**

Notes: BLOD = below limit of detection. Each individual sample was spiked with only one element (at an approximate concentration of 100 ppm) and analyzed for all the target elements for interference.
Acceptance criteria: The procedure must be able to unequivocally assess each target element in the presence of components that may be expected to be present, including other target elements and matrix components.

Table 9

Precision: Repeatability (round tablets)

Sample ID	Cd (ppm)	Pb (ppm)	As (ppm)	Co (ppm)	V (ppm)	Ni (ppm)
Repeatability-1	1.07	1.26	3.15	10.85	21.01	42.88
Repeatability-2	1.01	1.55	3.09	10.52	21.77	42.88
Repeatability-3	1.08	1.11	2.98	10.57	21.23	42.02
Repeatability-4	0.98	1.10	3.13	10.61	21.69	43.85
Repeatability-5	0.94	1.24	3.13	9.57	20.88	41.81
Repeatability-6	1.07	1.04	2.92	10.73	22.33	42.53
Mean	1.03	1.22	3.07	10.48	21.49	42.66
Standard devisation	0.06	0.18	0.09	0.46	0.55	0.73
%RSD	5.61	15.14	3.08	4.38	2.54	1.71

Notes: Six individual samples (tablet sets) were prepared at the 1J (5-gram dose) concentration containing the target elements. Acceptance criteria: %RSD ≤ 20% for each target element.

Table 10

Precision: Repeatability (round tablet sets)

Sample ID	V (ppm)	Co (ppm)	Ni (ppm)	Hg (ppm)	Pb (ppm)	As (ppm)	Cd (ppm)
Nominal 1J, 1 gram	100	50	200	30	5	15	5
Nominal 2J, 5 grams	40	20	80	12	2	6	2
Nominal 1J, 5 grams	20	10	40	6	1	3	1
Nominal 0.5J, 5 grams	10	5	20	3	0.5	1.5	0.5

Nominal concentrations for the target elements at the respective dosage

Sample ID	V (ppm)	Co (ppm)	Ni (ppm)	Hg (ppm)	Pb (ppm)	As (ppm)	Cd (ppm)
Round 1J, 1 gram	96.8 (2.5)	50.0 (3.0)	197.8 (3.1)	33.3 (4.2)	5.0 (1.9)	15.1 (0.8)	5.4 (2.7)
Round 2J, 5 grams	40.1 (2.7)	19.7 (2.8)	79.7 (1.4)	13.5 (2.9)	2.0 (6.1)	6.0 (2.5)	1.9 (8.3)
Round 1J, 5 grams	20.5 (4.4)	9.5 (5.3)	40.4 (3.2)	5.4 (4.5)	1.0 (6.7)	3.1 (0.6)	1.1 (2.7)
Round 0.5J, 5 grams	9.8 (4.9)	4.6 (3.8)	20.8 (1.5)	4.1 (3.6)	0.5 (40.7)*	1.6 (8.1)	0.4 (37.1)*

Notes: Experimental repeatability data on round tablet sets analyzed against the calibration curve for oblong tablet sets. Results are reported for triplicate analyses at each concentration. Numbers in parentheses are %RSD.
*The high %RSD represents the limit of quantification at the concentration containing the target elements under conditions analyzed.

Table 11

Comparison of random versus induced on-edge orientation of tablet sets

Sample ID	V (ppm)	Co (ppm)	Ni (ppm)	Hg (ppm)	Pb (ppm)	As (ppm)	Cd (ppm)
Random	40.1	19.7	79.7	13.5	2.0	6.0	1.9
On-edge	37.4	19.5	79.1	13.8	2.0	6.1	1.9
% deviation	-6.7	-1.0	-0.8	2.2	0.0	1.7	0.0

Notes: Experimental repeatability data were gathered on round tablet sets that were randomly oriented and preferentially oriented. Average results are reported for triplicate analyses, and the % deviation of preferred orientation were compared to random orientation.

accuracy of the results and no significant difference in reproducibility was detected (Table 10). To further test the robustness of the tablets-in-a-cup method of analysis, sets of round tablets previously analyzed were prepared with a purposely induced orientation, as shown in Figure 3. No significant differences were observed between the randomly oriented tablet sets and tablet sets that were oriented on edge (Table 11).

FIGURE 3

Induced orientation of tablets

a. On-edge orientation

b. Flat orientation

Conclusions

The results reported here demonstrate that the WD-XRF technique has huge potential for analyzing finished pharmaceutical dosage forms according to ICH Q3D and USP <233>. In contrast, most labs that use ICP techniques focus their control strategy on raw materials and processes as a means of assessing risk and they avoid routine testing of final dosage forms. Implementing WD-XRF—an easy-to-operate, nondestructive, and direct analysis technique—enables lab personnel to conduct final testing that ensures all products meet safety requirements. Furthermore, because sample preparation is easy—simply adding tablets to a cup—WD-XRF can be applied in a manufacturing environment with minimal setup. *T&C*

References

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