PANalytical

EPSILON 5

Analysis of metals in pharmaceuticals

Introduction
The pharmaceutical industry is heavily regulated beyond all other industries because many of the materials produced are potentially dangerous and can be severely toxic if not manufactured with proper care. This level of regulation has led to the mandatory examination of all substances from starting materials to finished products. Generally this includes the proof of product stability, drug-release profiles, Active Pharmaceutical Ingredient (API) form, API quantification, and product safety, for example, sterility (for non-biological materials) and the presence of foreign materials including heavy metals.

One of the most common product safety related analytical tests is the quantification of inorganic impurities within a pharmaceutical product. This normally includes the toxic metals, often referred to as heavy metals, such as As, Hg and Pb. Other metals, such as Fe, Cr, Ni and Zn are also referred to as heavy metals and have well documented health risks. Many APIs are synthesized using metal catalysts, which are invariably left bound to the final product. Therefore it is also common practice to measure residual metal catalysts, such as Ru, Pd and Pt, which are also well classified toxic elements. Throughout the manufacturing processes there are many potential sources of contamination. Therefore, in addition to measuring the starting materials it is essential to measure all finished products and in some cases intermediates, to demonstrate the process-recovery efficiency and compliancy with the various regulations.

Using energy dispersion X-ray fluorescence (EDXRF) it is possible to measure all (Na – U) elemental concentrations without heating or destroying the sample. Also, unlike time-consuming acid digestions, samples can be measured as loose powders or pressed into pellets and ready for measurement within minutes. XRF can measure larger sample volumes resulting in a better characterization of final products, which are often complex composites numerous excipients and APIs. Furthermore, the XRF technique provides high accuracy and precision with excellent detection limits (0.1 – 1 mg/kg).

Epsilon 5: the essential element
The Epsilon 5 is a fully integrated energy dispersive XRF analyzer consisting of a spectrometer, X-Y sample handler and software. It has a unique combination of features including a three-dimensional, polarizing geometry, together with a 600 W Gd anode X-ray tube and 100 kV generator, up to 15 polarizing and secondary targets and a high-resolution PAN-32 detector. Designed for accuracy, precision and sub-ppm detection limits the Epsilon 5 excels in the analysis of medium to heavy metals, targeting a wide range of elements that are of interest to health and pharmaceutical sciences.

Advantages of XRF

X-ray fluorescence spectroscopy benefits from simple, essentially hazard-free, sample preparation. Most other elemental analysis techniques require the sample material to be dissolved. This often involves different digestion steps/ recipes, requiring a wide variety of aggressive reagents and solvents. This is far more time-consuming and costly than the simple grinding and/or pressing sample preparation required for XRF analysis. While pharmaceutical matrices can vary widely, XRF is a robust technique which can readily measure multiple materials with a single set of calibration curves. XRF provides the advantage of large sample size capacity, minimizing any sampling or sample preparation errors. Materials can be measured directly in the 'as received' form, for example liquids, solids, or powders.

Method approach

The purpose of this method is to analyze a range of pharmaceutical samples with one robust calibration program. Two common excipients, lactose and wood-cellulose were chosen to set up the calibration.

A challenge for trace element analysis is making accurate corrections for variations between different sample matrices and sample thickness that affect element and background sensitivities. Fortunately a type of X-ray scatter peak, the Compton peak, is directly related to the sample matrix composition and can be used to make robust, accurate corrections. The most useful Compton peaks used for the sample matrix corrections are associated with specific wavelengths used to excite the sample. In the Epsilon 5 sample excitation is performed via multiple secondary targets, which have different excitation wavelengths.

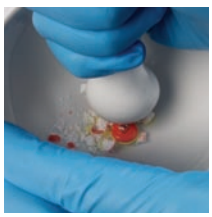
Since the Epsilon 5 can accommodate up to 15 secondary targets it has the flexibility required to handle such a challenging application, as there is always a Compton line close to the analyte line of interest.

Preparation of standards
A common excipient material (pharmaceutical grade wood-cellulose) was chosen as a base material. In-house standards were prepared using ultra-pure commercially available organo-metallic standards representing 6 heavy metals. The elements were chosen to represent a significant proportion of the periodic table ranging from light to heavy transition metals (Cr to Pt). Standard chemical techniques were employed to dope the cellulose and produce a set of standards. The standard concentrations were confirmed by ICP-MS.

Sample preparation
All materials, standards and samples, were analyzed as loose powders (approximately 2500 mg) in liquid sample cups. This simplified process reduces sampling and sample preparation errors often compounded when using small masses and dissolution procedures commonly employed by other techniques.

Solids






Liquids




Measurement criteria
Calibration curves were set up using different conditions with a total measurement time of 30 minutes (real time). The excitation parameters used to cover the whole range of elements from Ti to Pb are shown in Table 1.

If the analysis of few elements is required, some condition sets may be removed, thereby reducing the measurement time.

Condition set	kV	Automatic mA adjustment	Secondary target	Filter	Elements (analytical line)	Measuring time (live*) s
1	75	Yes	Ge	None	Cr *Kα*, Ni *Kα*	300
2	100	Yes	Zr	None	As *Kα*, Pt *Lα*	300
3	100	Yes	Al_2O_3	Zr	Ru *Kα*, Pd *Kα*	300
4	100	Yes	CsI	None	Compton	60

Table 1: Measurement parameters used in this study
Live Time* is the electronic counting time per condition set.

Performance
The Epsilon 5 software includes a very powerful deconvolution algorithm, which analyzes the sample spectrum and determines the net analyte peak intensity. The accuracy with which this is carried out is essential to trace element analysis. A spectrum of a standard material containing Ru (21.1 µg/g) and Pd (20.6 µg/g) is shown in Figure 1. This exemplifies the low background and excellent peak fitting (deconvolution) capabilities of the Epsilon 5.

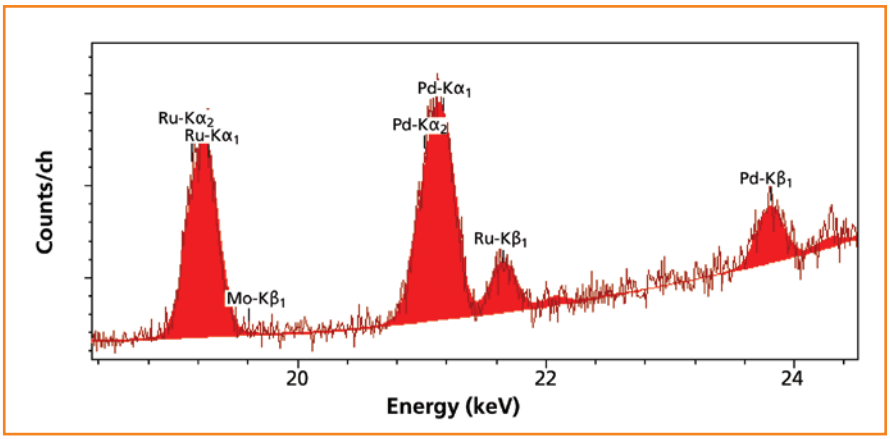


Figure 1: Spectrum of a standard containing 21.1 µg/g Ru and 20.6 µg/g Pd

Calibration
The method produced highly linear calibration curves; see Table 2 and Figures 2 - 5. Net elemental intensities were ratioed to the intensity of the Compton scatter peak to correct for sample matrix and mass or thickness variations (Table 2).

Element	Compton scatter peak	Concentration range (µg/g)	RMS	Correlation
As	Zr	0.0 - 22.7	0.0618	0.9999
Cr	Ge	0.0 - 20.7	0.2138	0.9993
Ni	Ge	0.0 - 132.6	0.7387	0.9999
Pd	Cs	0.0 - 20.6	0.2386	0.9999
Pt	Zr	1.2 - 21.3	0.1844	0.9994
Ru	I	0.0 - 21.1	0.3516	0.9999

Table 2: Calibration coefficients (measured using 300 second condition times)

Calibration graphs (Figures 2 - 5) demonstrate the capability of Epsilon 5 in determining heavy metal contaminants from various sources, such as environmental (As), stainless-steel reaction vessels (Cr) and catalyst residuals for the manufacturing of APIs (Ru and Pd).

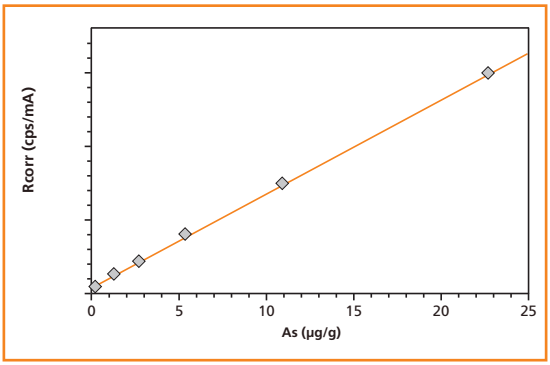


Figure 2. As calibration

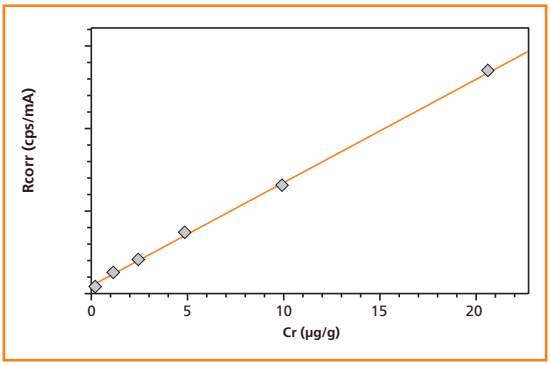


Figure 3. Cr calibration

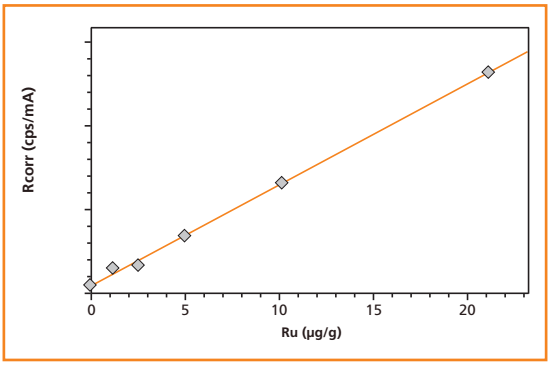


Figure 4. Ru calibration

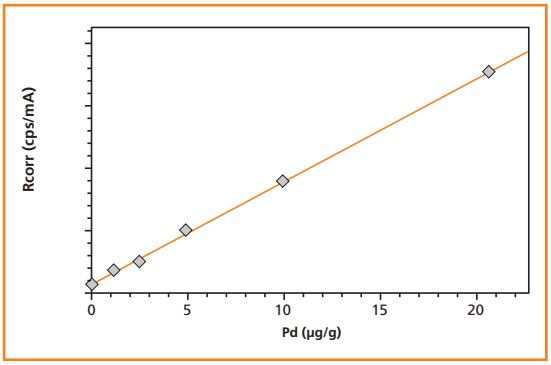


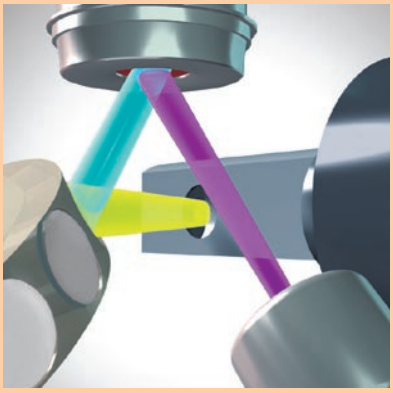
Figure 5. Pd calibration

Advantages of Epsilon 5

Epsilon 5 has several advantages over wet chemical based techniques and traditional EDXRF when it comes to the analysis of pharmaceutical materials:

- **Significantly reduced laboratory waste stream**
- **Unattended and fully automated analysis**
- **Extremely low backgrounds resulting from a sophisticated polarized optical design (even for samples with a very low density (for example: cellulose or other organic materials) where the background scatter is usually very high)**
- **Low detection limits for important elements such as Cd, Ag and Sb. This is achieved by using a 100 kV Gd X-ray tube, polarized optics and a Ge detector**
- **Highly robust calibrations are readily created. This is the result of powerful spectrum deconvolution which corrects for line-overlaps and even for unforeseen elements occurring in routine samples**

These advantages make Epsilon 5 the ideal system for analyzing the complete range of pharmaceutical materials.



Accuracy
The calibration root mean square (RMS) values presented in Table 2 demonstrate a high degree of accuracy for the method. The RMS value is a measure of the difference between the calculated concentration and the chemical concentration and is therefore a measure of the accuracy of the method. RMS values are often referred to as standard deviations and are dependant upon the range of concentrations in the calibration.

Precision
The precision of the Epsilon 5 for this application was demonstrated by analyzing a single standard against the calibration curve 10 times consecutively, Table 3. The statistical evaluation of the repeated measurements demonstrates excellent precision (standard deviation) for all elements. The average calculated concentrations were very close to the reported ICP-MS values, demonstrating the excellent agreement between the two techniques.

Repeatability (10 measurements)						
Element	As (µg/g)	Cr (µg/g)	Ni (µg/g)	Pd (µg/g)	Pt (µg/g)	Ru (µg/g)
Standard #3	5.53	4.92	33.53	4.85	5.48	5.05
Standard #3	5.49	4.97	33.74	4.49	5.01	4.65
Standard #3	5.55	4.75	33.43	4.37	5.26	5.23
Standard #3	5.60	4.75	33.59	4.93	4.77	5.14
Standard #3	5.35	5.04	33.64	4.63	4.37	5.33
Standard #3	5.57	4.96	33.09	4.91	4.69	5.36
Standard #3	5.48	4.67	33.11	4.66	5.38	4.84
Standard #3	5.63	4.80	32.88	5.02	5.28	5.23
Standard #3	5.48	4.52	32.79	4.71	5.03	5.32
Standard #3	5.39	4.89	32.77	5.06	5.22	4.95
Average	**5.51**	**4.83**	**33.26**	**4.76**	**5.05**	**5.11**
Standard deviation (RMS)	**0.09**	**0.16**	**0.37**	**0.23**	**0.35**	**0.24**
ICPMS	**5.40**	**4.90**	**33.50**	**4.90**	**5.00**	**5.0**

Table 3: Replicate analyses of standard #3

Detection limits
Typical detection limits of elements in two common excipient materials, cellulose and lactose, are listed in Table 4. Note these values can vary slightly depending upon the sample matrix (the composition and presence of line-overlaps). Unlike wet chemical extraction-based techniques, which often suffer from inefficiencies due to metal-organic complexation, Epsilon 5 achieves excellent detection limits for elements in either excipient, regardless complexation.

The lower limits of detection (LLDs) are calculated from three times the standard deviation of twenty repeated measurements of a blank sample. An advantage of this method is the capability to achieve lower detection limits by simply extending the measurement time. This is shown in Table 4 where the application measurement time was extended (from 30 minutes to 80 minutes real time) to achieve lower detection limits for As, Pt, Pd and Ru.

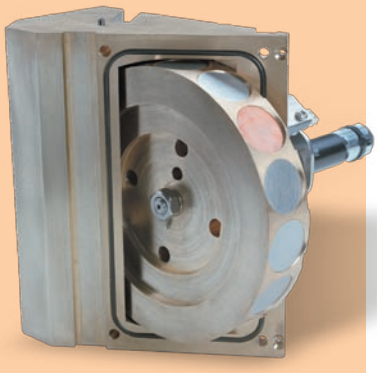
Calculated lower limits of detection in µg/g								
Excipient	Application time (min)	Measurement time (live*) s	As	Cr	Ni	Pt	Pd	Ru
Lactose	30	300	0.12	0.36	0.15	0.48	0.48	0.60
	80	1200	0.06	NA	NA	0.24	0.24	0.30
Wood-cellulose	30	300	0.18	0.60	0.12	0.48	0.72	0.36
	80	1200	0.09	NA	NA	0.24	0.36	0.18

Table 4: Lower limits of detection
Live Time* is the electronic counting time per condition set (see Table 1).

Analytical flexibility

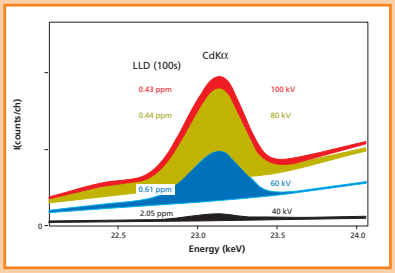
The Epsilon 5 can be 'tuned' to get the lowest detection limits for a large number of elements. This flexibility is achieved using a set of programmable polarization and secondary targets. Up to 15 targets can be mounted in the Epsilon 5. The basic system is configured with 9 targets, giving comprehensive coverage of the periodic table. The additional 6 target positions can be configured when optimum excitation conditions are required for the lowest possible detection limits in specific applications.

A cost-effective alternative – making X-ray tube changes for optimum performance a thing of the past.



High-voltage Gd X-ray tube

The PANalytical Gd X-ray tube has significant advantages for the analysis of heavy metals. Operating at a maximum power of 600 W and voltages between 25 and 100 kV it is unique among energy dispersive XRF spectrometers.
The characteristic tube lines of the Gd anode enhance the fluorescence of elements in the range rhodium to barium. Furthermore, the 100 kV capability has clear advantages for the excitation of heavy element K-lines, for example cadmium, as illustrated below.



Conclusions
Epsilon 5 is a unique instrument and is capable of non-destructively determining metal concentrations in pharmaceutical materials, with minimal sample preparation. This is possible because of its ability to generate high-energy polarized X-rays without producing heat that would normally damage these sample types. The applied method demonstrates the efficiency of Epsilon 5 to analyze a range of pharmaceutical matrices from excipients, APIs, intermediates and finished products. Furthermore, Epsilon 5 demonstrates a high degree of accuracy, precision and robustness necessary for validation and routine measurement of pharmaceuticals. The calculated detection limits (LLD) and analysis accuracy (RMS) clearly demonstrate that Epsilon 5 can reliably measure most elements of concern well below 1 µg/g (ppm). Thus Epsilon 5 can be used to meet the requirements of any international pharmacopeia and related regulatory bodies.

Epsilon 5 is fully capable of measuring other elements that are known to be of interest to biologists, food scientists, pharmacologists and health regulators such as Na, Mg, Fe and Co (essentially any element from Na to U). Also, the stability of Epsilon 5 is such that individual calibrations can be used for months, meaning that time-consuming re-standardizations are unnecessary and users can benefit from highly consistent data over the long term.

Equipment configuration of Epsilon 5		
Epsilon 5 energy dispersive X-ray fluorescence spectrometer and controlling software		
X-ray tube:	Type:	Side-window tube
	Anode:	Gd
	Window:	Be (150 µm)
	Rating:	25 - 100 kV, 0.5 - 24 mA,
		maximum power 600 W
	Internal water cooling	
Detector type:	PAN-32:	Ge X-ray detector
	Crystal:	30 mm^2, 5 mm thick
	Window	Be (8 µm)
	Energy range:	0.7 - 100 keV
	Resolution:	typically 135 eV, but ≤140 eV (2000 cps, Mn Kα)
	Liquid nitrogen cooling	
Polarizing optics:	3-dimensional design	
Targets:	Al, CaF_2, Fe, Ge, Zr, Mo, Ag, Ce_2O_3, Al_2O_3, KBr, CsI	
Diaphragm:	W	
Sample changer	133 position robotic sample changer	
Medium	Vacuum and helium	

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