Frequently asked questions (FAQs) about the analysis of elemental impurities in pharmaceutical drug products

Thermo Fisher Scientific has several hundred sales, technical support, service and applications support personnel globally tasked with supporting our customers' challenges with trace elemental analysis. We consolidated questions that our field team was receiving from customers about analysis of elemental impurities in pharmaceuticals and biopharmaceuticals following ICH Q3D guidelines and/or USP 232 & 233 methods.

What is an elemental impurity?

Elemental impurities are traces of metals that can be found in finished drug products. The analysis of elemental impurities is necessary to describe the concentration of the trace elements in final drug products. Some elements, such as arsenic (As) or lead (Pb), may be present in a drug product based on their natural occurrence. Others, such as palladium (Pd) or platinum (Pt) may be present as residues from catalysts which are used in active pharmaceutical ingredient (API) manufacture. Some elements are intentionally added to a drug product, whereas others leach into the drug product from the drug packaging, or, in the case of elements such as nickel (Ni) or copper (Cu), stem from manufacturing equipment. Due to their potentially adverse effects in the human body, and unknown effects on the stability and effectiveness of a drug product, the concentration of elemental impurities has to be determined in all drug products.

What is an inductively coupled plasma (ICP)?

In addition to solid, liquid and gas, plasma is sometimes considered as a fourth state of matter. A plasma is formed when a gas contains a significant amount of ions and free electrons. Plasmas can be high energy, high temperature entities with temperatures of up to 10,000 °C. An example of a high energy plasma is a lightning flash.

In an ICP as used in ICP- optical emission spectroscopy (OES) and ICP- mass spectrometry (MS) instruments, argon (Ar) is used to generate the plasma inside a torch assembly made from quartz. The required energy (typically 1500 W) is provided through an RF frequency and applied via an induction coil surrounding the torch. The plasma is ignited using a short electrical discharge and maintained as long as the RF frequency is applied. The sample is introduced through the central channel of the torch and hence directly entering the plasma. All parts of the system are either water cooled, or cooled using Ar at a higher flow rate.

What is the difference between ICP-OES and ICP-MS?

Both techniques have a similar excitation source for the sample, an inductively coupled plasma. In ICP-OES, the atoms and ions in the sample are excited by the energy provided by the plasma. After relaxation to the ground state, the atoms emit light of a characteristic wavelength, which is then separated in an optical system and detected by a photosensitive chip.

In ICP-MS, the sample is ionized and the ions are separated according to their mass/charge (*m/z*) ratio. Also here, each element gives a characteristic set of signals (the isotopic fingerprint). For both ICP-OES and ICP-MS the signal intensity for each element is directly proportional to its concentration in the measured sample.

Which instrument type should I choose, ICP-OES or ICP-MS?

Both techniques are suitable for meeting the United States Pharmacopeial Convention (USP) Chapter <232> concentration limits, but the sample preparation procedure may dilute the analytes of interest to concentrations below the detection limits of an ICP-OES. ICP-MS provides a larger dynamic range, which may be a requirement for certain applications.

Is ICP-OES better for samples with complex matrix?

It is true that ICP-OES instruments are more matrix tolerant than ICP-MS instruments. However, modern ICP-MS systems are much more tolerant to sample matrices with high levels of dissolved solids than their predecessors. As a rule of thumb, an ICP-MS can easily tolerate 0.2 % (m/v) total dissolved solids (TDS). If higher matrix loads are expected in the sample, the sample should be diluted to reduce potential matrix issues. Samples can be diluted using aerosol or liquid dilution techniques. Aerosol dilution is performed using Argon Gas Dilution (AGD) which involves the introduction of argon into the sample aerosol prior to reaching the plasma. Liquid dilution can be performed automatically using in-line dilution equipment and involves the addition of an appropriate amount of solvent. It should be noted that aerosol dilution requires that all standards and sample be diluted by a fixed amount. Liquid dilution allows every sample to be diluted by a factor appropriate for the analysis.

Is Atomic Absorption Spectroscopy (AAS) an option for analyzing elemental impurities in pharmaceuticals?

Although USP chapter <233> describes the two techniques ICP-OES and ICP-MS in detail, it also states that alternative techniques can be used if they can be validated according to the same acceptance criteria. Therefore, the use of Furnace AA would be also an option for the determination of elemental impurities. However, Flame AA is not suitable as the required detection limits for the ‘big four’ (arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb)) are too low for this type of instrumentation.

Which acid is best to prepare pharmaceutical samples?

Choice of acid is dependent upon the constituent of the drug e.g., if a silicone excipient is present, hydrofluoric acid (HF) will be required and special safety precautions should be followed*. Many people believe that nitric acid (HNO_3) is a universal solution which is not entirely correct. Nonetheless, nitric acid (maybe in combination with hydrogen peroxide) is a good acid to start with when organic material needs to be digested. Sulphuric acid (H_2SO_4) can be used as a means to increase reaction temperature relative to pressure. Addition of

hydrochloric acid (HCl) is often used to stabilize Hg and Pt group elements (PGEs). For more help with choosing an appropriate acid, consult the manufacturer of your closed vessel digestion system as they usually have a 'cookbook' available. Key to the use of the cookbook is knowing what is contained in the drug i.e., get a full listing of the excipients and APIs. Details of reagents for trace elemental analysis can be found here.

*Special safety precautions must be taken when using HF. Please refer to local guidelines to ensure all safety requirements are met.

What is the required acid purity that I should use in my laboratory?

The purity of the acids and reagents used is a crucial factor for the attainable method´s performance. For ICP-OES certified trace metal grade is usually sufficient whereas ultra-trace grade is required to leverage the full potential of your ICP-MS system. Also the purity of the water used to prepare diluents and dilutions is a limiting factor. If water (or any solution used in elemental impurities testing) should be stored for some time Teflon containers should be used in order to avoid contamination of the solutions. All glassware used for sample preparation should be well rinsed with water or dilute acid and the water/dilute acid should be of high purity so as not to introduce contaminants while cleaning the glassware.

What is the best choice for an internal standard?

Internal standards are used in many applications to correct for potential drifts in instrumental sensitivity over time or changes in the sample matrix. The selection of a suitable internal standard should include the following aspects:

- The internal standard should have a first ionization potential similar to the analyte, and should have a similar mass as the analyte.
- The internal standard must not be part of the sample.
- It should not generate or be affected by spectral interferences.
- It should be at a low and uniform (preferably zero) concentration in all samples.

Although ICP-MS is a technique considered by some to be relatively robust with respect to matrix effects; in reality matrix effects do commonly exist and the use of internal standards is standard practice. Internal standards also help account for changes in the transport efficiency of the sample through aerosol.

How much should I dilute my samples before analysis?

ICP-MS is a highly sensitive technique for the detection of elemental impurities. The required limits set out by USP chapter <232> are far higher than the levels that can be detected with an ICP-MS, so dilution can be accomplished easily without compromising performance. You should make sure samples are diluted such that all analytes are at concentrations well within the calibrated concentration ranges. It should also be noted that analytes shouldn't be diluted such that their concentrations are too close to the instrument's detection limit. It is important to remember that maintaining a clean instrument is a mandatory prerequisite to long-term performance of the machine. Hg, for example, is well known to show memory effects that increase wash out times or even lead to overestimation of the content of subsequent samples. It is therefore recommended to start with a higher dilution factor during method development and decrease if required.

How does the format of my sample impact dilution?

If samples are in solid form, the dilution incurred will be dictated by the digestion procedure being used to prepare the sample for analysis. If samples are being analyzed in their native form or after simple dilution, there may be more flexibility in the dilution factor used during preparation. Regardless of the sample's original form, the sample matrix must contain a tolerable level of dissolved solids prior to introducing it into the instrument. If an ICP-MS is being used for analysis, that means the sample matrix should contain 0.2 % TDS or, if no special configuration for the sample introduction system is used (e.g., utilizing AGD).

How is the method detection limit calculated and how is it related to the parameters instrumental detection limit and blank equivalent concentration?

The parameters BEC, LOD and LOQ are frequently used to describe the detection capabilities of an analytical instrument. The acronym BEC abbreviates the parameter blank equivalent concentration. This value refers to the 'apparent concentration', and is composed of the contamination level in the blank, any residual interference signal and the instrument background (from the detection system). The parameter instrumental detection limit (IDL, often referred to as Limit of Detection, LOD) is defined as the limit of detection that can be achieved by the instrument used. This amount is typically defined as a quantity that gives a distinguishable signal in the detection system. The common definition for the IDL is based upon the standard deviation (LOD = 3.3 x standard deviation of the regression line of calibration curve) of a blank sample measured in the beginning of a calibration curve, or a minimum signal to noise ratio of 3:1. Both parameters, BEC and IDL are automatically calculated by Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ (ISDS) platform software.

In contrast on the IDL, the parameter method detection limit (MDL) includes the extent of all dilution steps carried out during the sample preparation. The MDL is typically based upon a blank solution that has been prepared according to the preparation procedure that's being used to prepare all the samples. In situations where there is no sample preparation or preparation involves a single dilution step, MDLs might be calculated based on the standard deviation of a low level standard. MDL concentrations are always more conservative than IDL concentrations.

How much more difficult is it to learn ICP-MS in comparison to ICP-OES?

Modern ICP-MS instrumentation has become far easier to operate, both in terms of hardware and software. Furthermore, the advent of intelligent interference removal features such as 'low mass cut-off' and kinetic energy discrimination (KED) mode has more or less overcome the need to worry about which settings (cell gas, operation mode, cell operating parameters) are appropriate for which element. Overall, the barrier to success with ICP-MS has been lowered significantly and it should not be viewed as a technically challenging alternative to ICP-OES.

Is it also possible to run samples prepared in organic solvents on an ICP-MS or is it just an option for ICP-OES?

It is possible to run samples prepared in organic solvents using ICP-MS. In contrast to ICP-OES, some additional measures have to be taken to achieve optimum performance of the instrument. In particular, the ICP-MS instrument must be fitted with an organics kit which includes a mass flow controller for introducing an Ar/oxygen (O_2) mixture into the plasma, to burn up the carbon in the solvent to prevent it condensing on the cones.

Can slurry nebulization be used with pharmaceutical products?

Slurry nebulization is more commonly used in environmental testing applications. In principle yes, and there have been studies done on this, one can use it for pharmaceutical testing. Obviously particle size is a key factor here—the smaller the better. However, it is also worth pointing out that the method preferred by USP is microwave digestion.

What is a segmented flow sample introduction system and how can it help me?

Segmented flow sample introduction systems are a very helpful tool to increase the productivity in your laboratory. Such devices work with a valve system that enables fast uptake of the sample to the plasma, and minimize the wash out times between different samples. Therefore, the time required for analysis can be shortened considerably. An example for such a system is the sprint valve system available on the Thermo Scientific™ iCAP™ 7600 ICP-OES.

Can I use autodilution to help in the preparation of standards and samples?

Yes, autodilution can be used on both ICP-OES and ICP-MS to automate and streamline preparation of calibration standards and perform final sample dilutions. Autodilution systems are also based on valve systems similar to segmented flow introduction systems and are capable of generating different calibration curves (e.g., for drug products with varying daily dosage) from a single stock solution. This reduces the workload for laboratory personnel, and also reduces the amount of human interaction with the samples. If a given sample exceeds the calibration range or leads to an internal standard response outside the allowed acceptance criteria, a sample can be automatically diluted and the analysis is repeated. At the same time, autodilution systems are

completely integrated into the Qtegra ISDS operating software, so that all dilution (prescriptive or automatic) steps are documented in a compliant software environment.

Can I use ICP-OES and ICP-MS as a screening tool?

Both ICP-OES as well as ICP-MS can acquire full spectra to identify all elements present in a sample. This function is not only useful for method development, where it helps to identify the origin of interferences, it can also be used as part of a risk based testing approach. If suppliers issue certificates for given elements, these can be removed from quantitative testing, and instead be systematically screened for in a spot testing approach to assure compliance at all times.

What other effects may compromise my results?

Interferences are of most concern in ICP-MS. One can basically distinguish two types of interferences: spectral interferences and physical interferences. Mostly, polyatomic interferences are observed, but can mostly be efficiently suppressed by using an instrument equipped with a collision/reaction cell system.

Other interferences observed are isobaric interferences, for example, caused by two elements present in a sample that have isotopes with a common mass number. Mathematical correction using another isotope of the interfering element and calculating the contribution to the signal by means of the isotopic abundance is a way to overcome these interferences.

Doubly charged ions can interfere with some elements, especially if a high concentration of an element with a low 2nd ionization potential is present in a sample, e.g., samarium (Sm), a member of the rare earth elements, that would interfere with the detection of As at *m/z* 75.

In addition, ionization effects may lead to false positives, for example, if carbon is present in a sample (e.g., after direct dissolution of a sugar containing product in water), As may show an elevated response. Microwave digested samples are normally not affected.

In which instances would a triple quadrupole instrument be required for testing of elemental impurities?

Triple quadrupole ICP-MS instruments use an advanced interference removal mechanism and are capable of achieving LOD for some elements in challenging sample matrices. For typical samples as they are found for elemental impurities analysis, single quadrupole instruments are perfectly capable of removing all typical interferences. However, triple quadrupole ICP-MS offers advantages for the analysis of heavily interfered analytes, such as phosphorous (^{31}P) or sulphur (^{32}S), and can therefore be a valuable tool when used in research-focused laboratories.

We have recently purchased an ICP-MS from Thermo Fisher Scientific—can I connect my existing chromatography systems to it for speciation?

Yes, the interfacing of chromatography to our ICP-MS products is straightforward. In principle, the column ending is connected directly to the nebulizer of the ICP-MS system. Qtegra ISDS software contains a plug-in for Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software in order to seamlessly integrate and control Thermo Scientific ion chromatography (IC), ultra high performance liquid chromatography (UHPLC) or gas chromatography (GC) products. In this case, creation of the method and the sample list can be accomplished in one single software window, with automatic triggering of data acquisition. For chromatography systems from other vendors, a trigger cable can be used to start and stop acquisition.

Is there any instrument that can measure the heavy metals in raw materials? Solids?

Laser ablation (LA) is a tool for the direct analysis of solid materials with ICP-MS. There are however some limiting factors to the use of LA-ICP-MS for the direct analysis of solid material, for example the homogeneity of the sample. Furthermore, the sample needs to resist the impact of the laser beam without decomposition.

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Will ICP-OES detection limits be capable of handling the 'big 4' in final product when the permitted daily exposure (PDE) is higher than 10 g·day^{-1}?

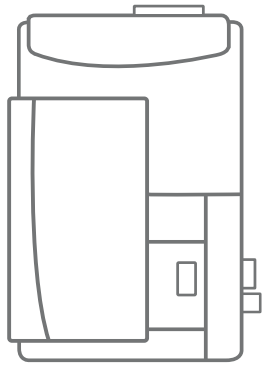
In general, ICP-OES is perfectly capable of achieving the required detection limits of USP chapter <232>. However, there can be two limiting factors: Due to the definition of PDE values for individual elements, the larger the daily dose is for a given drug product, the lower is the detection limit required. There are also ways to improve the detection capabilities of an ICP-OES in cases where more sensitivity is required, e.g., by means of a hydride generation system. Such a system can typically increase the detection sensitivity for e.g., As and thus helps to still achieve the required detection limits.

Which ICP-OES and ICP-MS instruments are available from Thermo Fisher Scientific?

Thermo Fisher Scientific has over thirty years of experience in designing and manufacturing ICP systems. We offer both ICP-OES and ICP-MS products. Our ICP-OES products offer both dedicated radial and dual radial/axial views. In our ICP-MS portfolio we offer both single and triple quadrupole mass spectrometers together with high-resolution magnetic sector mass spectrometers:

- Thermo Scientific™ iCAP™ 7000 Plus ICP-OES Series
- Thermo Scientific™ iCAP RQ™ ICP-MS
- Thermo Scientific™ iCAP™ TQ ICP-MS
- Thermo Scientific™ ELEMENT™ 2 High-Resolution ICP-MS Series
- Thermo Scientific™ ELEMENT™ XR High-Resolution ICP-MS

The iCAP RQ ICP-MS is the recommended model for pharmaceutical elemental impurities.



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