



White Paper

Preparation of Pharmaceutical Samples for Metals Analysis

Alan Cross

Technical Specialist, Metals Laboratory



Preparation of Pharmaceutical Samples for Metals Analysis

There has been a lot of discussion regarding the methods of analysis for pharmaceutical products as we await the upcoming changes to both the EP and USP, relating to the analysis of metal impurities.

The USP chapter <233> currently offers two compendial techniques for the analysis of these impurities; Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). These techniques are also referred to in the EP section on metal residues, along with several other techniques.

The relative virtues of ICP-MS and ICP-OES have been frequently discussed, especially with respect to detection and specificity parameters. However, sample preparation is often overlooked as an important aspect of the analysis of pharmaceutical samples. After all, if the sample is not prepared properly, any subsequent analysis will be compromised. Both the EP and USP do cover aspects of sample preparation, but not in any great detail. The EP gives guidance as to whether a sample is suitable for analysis by following a flow chart whereas the USP lists four techniques for preparation, namely direct analysis, dissolution in aqueous, dissolution in organic solvents, or closed vessel digestion. Each of these techniques has its own advantages and disadvantages.

In an ideal world, all samples would be in a state that meant they could be aspirated directly into the instrument for analysis. If this were possible there would be no danger of losing volatile analytes, of sample contamination or of worries about detection limits due to dilution of the sample, as discussed in this white paper. Unfortunately samples rarely come in this condition, and even if they do there are other factors to consider that may mean that analysis is not necessarily simple. Nonetheless, direct analysis is sometimes an option.

Direct Analysis

One big consideration for this technique is the effect of total dissolved solids (TDS). Plasma techniques (i.e. ICP) are particularly sensitive to TDS and so the presence of TDS can be detrimental to the quality of the analysis. Obviously tolerance to TDS varies greatly on instrument set-up, and different configurations of nebulisers and spray chambers can be used to optimise conditions. As an approximate guide, ICP-OES is more tolerant of TDS, with levels of 5% being routinely accommodated. In some special cases, levels of up to 20% can be tolerated. ICP-MS on the other hand is more vulnerable to interference from TDS, with a maximum level of around 0.2% being acceptable.

With a direct analysis approach, the level of TDS will often be higher than one would find in a prepared sample. These effects can be mitigated by simple dilution with the aim of minimising TDS levels, and, of course, this option is available for all sample preparations.

Another consideration of analysing direct samples is the effect of organic material on the behaviour of the plasma. As the organic material is consumed within the plasma, the chemistry alters, which often causes a shift in the ionization equilibrium, thereby affecting the behaviour of the analytes. This phenomenon is particularly marked for analytes such as arsenic and selenium (due to the high ionization potentials for these elements), but can be overcome by the addition of an appropriate amount of an organic solvent such as methanol or butanol to the calibration standards in an attempt to matrix match the samples to standards. There may also be a variety of instrumental variations, such as addition of oxygen to the plasma, or use of gas-mode analysis, which can overcome these problems. Another practical option is to carry out a standard addition calibration, where the calibration solution is added to the sample and the concentration of analyte is extrapolated from the obtained values. In any case the conditions for analysis should be evaluated as a part of any feasibility testing.

Dissolution

As mentioned above, dissolution of the sample is the next possibility for sample preparation. This technique again has similar considerations as direct analysis, but in a way can be a lot more controlled as the same solvents can easily be used for the sample and standards, thus negating the problems of unmatched matrices. Again, there will have to be a certain level of compromise with the dissolution of the sample to ensure the level of TDS is acceptable for the analysis technique used, whilst making sure that the analyte (if present) is measurable and above the level of quantitation (LOQ) of the instrument. To put it simply, dilution might push the elements of interest below the limit of detection of the instrument.

Closed Vessel Digestion

In the case of insoluble materials, closed vessel digestion will be required to destroy the sample matrix. This technique is especially valuable when analysing final products and some excipients. Microwave digesters are commonly used to perform the digestion as these are easy to use and can rapidly process many samples at a time. There are many different types of microwaves available on the market, each with their own advantages and disadvantages.

Typically, an aliquot of sample between 0.1 – 0.5g will be placed into a polytetrafluoroethylene (PTFE) or glass vessel along with acids. PTFE vessels will then be sealed with a tight fitting cap to create a pressurised environment, whereas glass vessels will be loosely covered and placed into a chamber which is then pressurised to generate the digestion environment. Once samples are digested, the digestate is transferred to a volumetric vessel and made to the required volume using high purity water.

If digestion is effective then the matrix effects from organic material should be sufficiently removed by

oxidisation (to carbon dioxide and water). Though the organic matter is effectively destroyed by the microwaving process there may still be TDS considerations to be taken into account, as these will not be destroyed by the microwaving process. This is an important consideration when planning on the sample size and final preparation volume.

The choice of acids used for the preparation of digested samples is also important. Typically nitric and hydrochloric acids are used for the preparation of these samples, often in combination with each other to optimise digestion conditions. The presence of hydrochloric acid is useful for stabilisation of platinoid group elements, but can sometimes cause unwanted side reactions (e.g. formation of insoluble silver chloride). The presence of chloride in the final test solutions can also be detrimental, especially for ICP-MS as the chloride contributes to polyatomic interferences (most notably ArCl for As and Se). These polyatomic interferences can generally be removed by use of collision or reaction gases within the ICP-MS after the ions have been generated by the plasma. Nitric acid and peroxide are often used for organic matrices as the peroxide is an effective oxidising agent and destroys the organic matrix, but care must be taken when testing for osmium as this can form volatile osmium oxides in the oxidising atmosphere which are easily lost from the sample.

In some cases hydrofluoric acid (HF) will have to be added to destroy certain materials such as titanium dioxide or silica if these have been used in the final product, or even if the excipient itself is being tested. In cases where HF is necessary, special instrumental adaptations need to be made to prevent damage to glass parts, either by direct replacement with PTFE materials or by use of specialist equipment that can generate a dry sample introduction. This preparation will also have an effect on the overall TDS levels, so it is important to consider this when looking at the preparation of these samples.

Obviously this is a very limited look at the available sample preparations, more an overview of the most likely methods for sample preparation. There are a huge variety of different options than can be used, but each must be carefully considered especially in terms of the preservation of analyte and minimisation of the effects of TDS and organic materials.

There can be some situations where preparation is less critical. For example, x-ray microfluorescence (XRF) can be used for elemental analysis but suffers inasmuch as it has higher detection limits than that of the plasma techniques. However, a sample can be analysed directly with no preparation.

Another option is to use laser ablation to generate a vapour directly from the surface of the sample, which is then swept into an ICP-MS. Again this is a technique that does not require sample preparation. However, it is not commonly used as the samples need to be calibrated using standards of a similar material and these are generally not readily available.

Specific considerations also apply to particular elements. When preparing samples for analysis of mercury, for example, care must be taken not to lose the analyte as it can be volatile. This is especially relevant when carrying out microwave digestion. That said, this volatility can be useful since there are mercury analysers on the market that measure levels by heating the sample and measuring the amount of mercury volatilised. These instruments are supremely practical as they can analyse the sample directly. That said, a single element technique is unlikely to be sufficient for complete compliance with pharmacopeial requirements.

In this overview, a brief critique of some of the sample preparation techniques that can be used for ICP analysis has been given. However, it must be said that it is important to evaluate both the preparation and analysis techniques together when considering how to assess elemental composition of pharmaceutical products.

Contract laboratories/contract research organisations (CROs) are a useful resource when developing testing regimes for pharmaceuticals and pharmacopeial testing. They will generally be able to pull from a wide pool of knowledge and resource to efficiently optimise preparation and analysis conditions. They can also validate methods according to regulatory guidelines or company specific requirements. Once validated this method can be transferred back to be performed in-house or samples may be sent to the CRO for routine analysis for batch release.



Alan Cross - Technical Specialist, Metals Laboratory

Alan graduated in 1999 from the University of Exeter with a Bachelor's Degree in Chemistry. He has worked across a variety of sectors including environmental, food, pharmaceutical and catalysis focusing mainly on analytical chemistry. During this time he covered a wide range of instrumental and wet chemistry techniques. Alan's main area of expertise is metals analysis covering ICP-MS, ICP-OES, AA and the related sample preparation such as microwave digestion and ash samples.

For further information please contact Alan +44 (0)118 9184000 or alan.cross@rssl.com