SPECTRO

Simultaneous ICP-MS in the Pharmaceutical Industry

A Powerful New Tool for Elemental Analysis

Mass spectrometry has become a popular and widely used technique in the pharmaceutical industry for the analysis and identification of organic compounds. The same basic principle can also be applied to elemental analysis, and ICP-MS, in which a mass spectrometer is coupled with inductively coupled plasma as the ion source, is one of the most sensitive techniques available for the detection and quantification of metallic impurities and other inorganic substances in pharmaceutical products. Such substances can be present in pharmaceutical products for a number of reasons, occurring as active ingredients, as impurities in raw materials and as contaminants. Some, particularly the "heavy metals", are known for their toxicity, and as such are tightly controlled by regulation. Because of the repetitive dosing involved in most treatment regimes, permitted levels have to take into account the cumulative exposure to toxic elements, driving down the Limits of Detection required from analytical procedures.

For quality control purposes, the ability to screen for a number of metallic elements at low levels may be required. On the other hand, in products such as dietary supplements, some elements may be present at relatively high concentrations.

The ideal analytical technique for the elemental analysis of pharmaceutical products combines the ability to measure a wide range of elements at trace levels and the wide dynamic range needed to handle higher concentrations. Samples may vary from raw materials, intermediates, process chemicals and solvents to finished products, so analytical techniques must be able to handle a wide range of sample matrices. Any procedure used must also comply with the quality protocols used in the pharmaceutical industry such as US FDA 21 CFR Part 11. New proposed guidelines from the US Food and Drug Administration (FDA) describe elemental impurity limits in pharmaceuticals and analytical procedures.

The two analytical techniques described in these guidelines are Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP-OES) and Inductively Coupled Plasma–Mass Spectrometry (ICP-MS). The new SPECTRO MS is the world's first fully simultaneous ICP-MS system. Simultaneous ICP-MS has a number of advantages over conventional instrumentation, and this paper describes the design of this revolutionary instrument and gives some examples of its application in the pharmaceutical industry. ICP-MS can also be combined with other techniques such as HPLC and IC: this paper deals only with stand-alone applications.

ELEMENTAL IMPURITIES IN PHARMACEUTICALS - BACKGROUND AND REGULATION

The United States Pharmacopeia (USP) is the official public standards-setting authority for pharmaceutical products manufactured or sold in the United States. The USP sets standards for the chemical composition of prescription and over-the-counter medicines and other healthcare products and also for food ingredients and dietary supplements. USP standards are widely adopted worldwide.

Inorganic impurities in pharmaceutical products can originate from several sources:

- Impurities in raw materials or reagents
- Contaminants introduced during the manufacturing process, perhaps by leaching from pipes and other equipment
- Process residues such as catalysts
- Naturally occurring elements from plant or mineral sources
- Leachable metallic impurities in packaging materials

For many years, levels of toxic metal impurities in pharmaceutical products have been controlled using the Heavy Metals Test described in chapter 231 of the USP National Formulary (USP-NF). This test, involving classical chemistry and visual interpretation, is now considered to lack the sensitivity and specificity to adequately monitor these metals, so the USP has proposed new chapters 232 and 233,

respectively defining revised limits and test procedures that reflect the analytical technology now available, for probable implementation in May 2014.

The publications of these chapters have gone through several revisions, the latest dated from Feb. 1st, 2013. It is clearly stated in the introduction of Chapter 232, that element impurity levels present in drug substances and excipients must be known and reported.

Absolute metals concentrations in individual materials and components are clearly of less significance than their toxicity and the amount available to the subject, which depends on the dosage and frequency of administration and also on the "bioavailability" of the substance, which will be influenced by the method of administration. For example, drugs given orally are considered to be less "bioavailable" than those administered parenterally, e.g. by injection or intravenously. The basic limit is the Permissible Daily Exposure (PDE). When drugs are to be administered parenterally, and the total volume of liquid exceeds 100 ml, as with a drip, a limit is set on the absolute level that may occur in any component of the solution. This is referred to as the LVP (Large Volume Parenteral) Component Limit.

Inorganic arsenic and methyl mercury need to be determined by a speciation procedure if the total amounts determined exceed the limit.

It was mentioned above that parenteral administration is considered more efficient than via other routes.

In the update of the publications of chapters 232 and 233 dated May 2011, the permissible daily exposure PDE was multiplied with an exposure factor to reflect the different bioavailability for the different administration pathways. This has changed with the newest revision. The general exposure factors have been eliminated and PDE's are listed for three different administration pathways. The limits listed under oral daily dose apply also to the previously mentioned mucosal and topical routes of administration. All these limits are listed in Table 1. Manganese is not listed anymore compared to the version from May 2011. All limits in tables 1-3 are based on calculations for a person of 50 kg bodyweight, may derive from different studies and use different safety factors. It will be noted that many of the metals listed are Platinum Group Metals (PGM's). These metals are excellent catalysts, widely used in drug synthesis and as such potentially present in the finished product as catalyst residues. Note that some of these metals can be encountered as active drug ingredients rather than as contaminants: for example, the platinum compounds Cis-platin and carboplatin are widely used in cancer therapy and the potential for ruthenium compounds is also being explored. Gold and silver are other metals that can be encountered in pharmaceutical chemistry. The Rare Earth Elements (REE's) are beginning to find pharmaceutical applications. For example gadolinium has been used for some years in chelated form as a contrast agent in Magnetic Resonance Imaging (MRI).

Table 1: USP limit values for metallic contaminants in pharmaceuticals for the different routes of administration (Update Feb 2013)

Element	Oral Daily Dose PDE [a] [µg/day]	Parenteral Daily Dose PDE [µg/day]	Inhalation Daily Dose PDE [µg/day]	LVP Component limt [µg/g]
Cadmium	25	2.5	1.5	0.25
Lead	5	5	5	0.5
Inorganic Arsenic [b]	1.5	1.5	1.5	0.15
Inorganic Mercury [b]	15	1.5	1.5	0.15
Iridium	100	10	1.5	1
Osmium	100	10	1.5	1
Palladium	100	10	1.5	1
Platinum	100	10	1.5	1
Rhodium	100	10	1.5	1
Ruthenium	100	10	1.5	1
Chromium	--- [c]	--- [c]	25	--- [c]
Molybdenum	100	10	10	1
Nickel	500	50	1.5	5
Vanadium	100	10	30	1
Copper	1000	100	100	10

[a] PDE = Permissible Daily Exposure based on a person of 50 kg

[b] See speciation section

[c] Not a safety concern

Value decreased compared to May 2011

Value unchanged compared to May 2011

Value increased compared to May 2011

A speciation section is mentioned for Arsenic and Mercury. The arsenic limits are based on the assumption that all arsenic in the sample is of inorganic nature, the most toxic form. If the result for total arsenic exceeds the limit, a procedure that quantifies the different forms may show that the inorganic form meets the limit. The mercury limits are based on the inorganic II+ oxidation stage. The most toxic methyl mercury form is rarely an issue in pharmaceutical products. Limits for products that have the potential to contain methyl mercury (e.g. material derived from fish) are to be provided in the monographs.

In Europe the European Medicines Agency (EMEA) sets standards for metals used in drug synthesis via its

Committee for Medicinal Products for human use (CHMP).

The proposed and expected PDE limits are listed in Table 2. The color scheme in the table indicates that only a few elements and limits are the same in both approaches, some limits are higher in the USP, others in the ICH regulations. The ICH regulation also lists an additional 13 elements with limits;12 for inhalation. The USP limits are expected to be implemented May, 2014, the ICH limits later. The seemingly independent implementation of the ICH and USP implementation is of a concern in the industry. The Industry has organized a 'Coalition for the Rational Implementation of the USP Elemental Impurities Requirements' to evaluate 'what is a realistic timeframe for the implementation of both the USP and the ICH Q3D requirements based on the PDEs and concentration limits in the [ICH Q3D] pre-Step 2 document' [1].

Dietary Supplements are also treated as pharmaceutical products in this context, and chapter 2232 of the USP guidelines gives limits for elemental contaminants in these products and individual components. These limits are listed in Table 3.

Table 3: USP limit levels in Dietary Supplements

Element	Individual Component Limit (µg/g)	PDE (µg/day)
As (inorganic)	1.5	15
Cd	0.5	5
Pb	1.0	10
Hg (total)	1.5	15
Methylmercury (as Hg)	0.2	2

In some formulations metallic compounds may be present in bulk as fillers and similar ingredients. While not governed by this legislation, these elements may need to be measured as part of normal quality control procedures. Another important application of elemental analysis is for the monitoring of the waste water from pharmaceutical production activities, which could contain heavy metals or catalysts and contaminate the environment. Production is not the only source of pharmaceuticals entering the environment:

Table 2: Expected PDE limits based on the proposal of the International Conference of Harmonization ICH Q3D

Metal	Class	Proposed Oral PDE µg/day	Proposed Q3D Parenteral PDE, µg/day	Proposed Q3D Inhalation PDE, µg/day
As	1	15	15	15
Cd	1	5	5	5
Hg	1	50	5	5
Pb	1	5	5	5
Ag	2	3000	500	50
Au	2	10	10	5
Co	2	100	10	5
Ir*	2	500	50	5
Mo	2	50	5	500
Ni	2	2000	100	5
Os*	2	500	50	5
Pd	2	100	10	5
Pt	2	500	50	5
Rh*	2	500	50	5
Ru*	2	500	50	5
Se	2	200	100	100
Tl	2	10	5	5
V	2	100	10	5
W	2	40000	20000	5000
Al	3	50000	Different regional regulations	5000
B	3	2000	2000	1000
Ba	3	10000	1000	500
Cr	3	10000	1000	10
Cu	3	1000	100	15
Li	3	1000	500	25
Sb	3	1000	500	25
Sn	3	6000	500	50
		lower USP limit	higher USP limit	
		same USP limit	Element not in USP	

* *Insufficient data to establish an appropriate PDE; the PDE was established based on platinum PDE*

Class 1 *Metal impurities are toxic across all routes of administration, require consideration across all potential sources of metal impurities*

Class 2 *Metal impurities are toxic across all routes of administration, require assesment across all potential sources of metal impurities for V, Mo, W, Se, Ni and Co requireassesment only if intentionally added for Au, Tl, Pd, Pt, Ir,Os, Rh, Ru and Ag*

Class 3 *metal impurities with low toxicity by oral administration but require consideration for other routes of administration*

It should not be forgotten that residues or metabolites of pharmaceuticals administered to patients or livestock may be excreted and thus reappear in the environment. It has been reported, for example, that when Gd has been used as an MRI contrast medium, elevated levels of this rare element have been found in the water in the vicinity.

All these investigations require very high analytical sensitivity and specificity. There may also be the requirement to track drug metabolites during clinical trials, and this may mean the analysis of large numbers of samples.

Recommended Techniques

Two instrumental techniques are discussed in chapter 233 of the USP National Formulary, namely Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES) and Inductively Coupled Plasma – Mass Spectrometry (ICP-MS). Correctly implemented and used, both techniques have the potential to deliver the performance characteristics needed for pharmaceutical analysis:

- Wide range of elements detected
- High sensitivity
- Wide dynamic range
- Tolerance of different matrices, especially organics
- Low levels of interferences
- Ability to couple to separation techniques (e.g. HPLC, CE, IC) for speciation studies etc.
- Speed of analysis

SPECTRO ICP-OES instruments exhibit these characteristics, and their use in pharmaceutical analysis is described in the SPECTRO white paper "Metallic Elements in Pharmaceuticals", which can be found at www.spectro.com.

ICP-MS shares the same attributes, but exceeds the performance of ICP-OES in sensitivity, having detection limits in the parts-per-trillion range (ng/kg). Also, as will be explained later, the relative simplicity of mass spectra compared with optical emission spectra can ease interpretation and reduce the risk of inter-element interferences. It should also be remembered that ICP-MS detects the isotopes of each element, which in itself can be useful when using stable isotopes as tracers during pharmacological investigations. The new SPECTRO MS has a further advantage, in that it is the world's first fully simultaneous ICP-MS. This has several practical benefits over conventional designs, like full spectrum availability and isotope precision which are best appreciated by comparing the different systems currently in use.

ICP-MS - THE BASICS

An ICP-MS spectrometer works by separating ionized atoms according to their mass/charge ratio. In conventional ICP-MS, a sample solution is converted into a fine aerosol via a pumped nebulizer system, and introduced into a high temperature argon plasma where the analyte is atomized and subsequently ionized. These charged ions are then transferred into the mass spectrometer, where species of different mass/charge ratios are separated by magnetic and/or electrostatic fields and subsequently detected.
A basic ICP-MS comprises the main components shown in Figure 1.

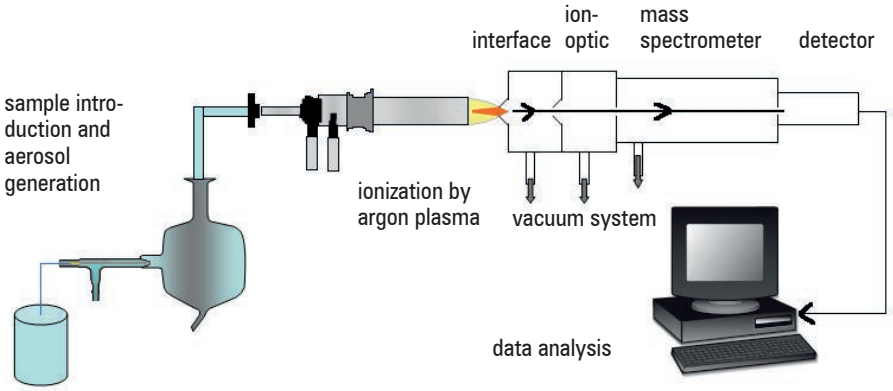

Figure 1: ICP-MS schematic

The system illustrated is designed for continuous measurements, i.e. the sample is introduced over a period of several seconds to minutes and the detector signal integrated over time. Noise and fluctuations in any of the system components will be superimposed on the detector signals and adversely impact the achievable Limits of Detection and/or reproducibility. Conventional MS detectors involve "scanning" the mass/charge ratio of the ions arriving at the detector over time, and while many efforts have been made to minimize the time interval between detection of individual masses, short-term fluctuations in the sample introduction system or the plasma can still lead to errors. For similar reasons, transient signals may not be accurately tracked, a problem known as signal "skew". In isotope studies, the abundances of two or more isotopes of interest have to be compared mathematically, so unless their concentrations are measured under exactly the same conditions, errors can be introduced. The only real solution is simultaneous and continuous detection of all masses of interest across the complete inorganically relevant mass range. In attempting to achieve this simultaneous detection using conventional technology, large, expensive, so-called "multi-collector" instruments (MC-ICP-MS) were designed that provided two or more movable detectors which could each be laboriously set to monitor a limited number of individual isotope masses. None of these instruments provided the ideal capability of simultaneously observing the whole mass spectrum. The new SPECTRO MS, utilizing an entirely new concept in detectors, is the first instrument to offer all the advantages inherent in the fully simultaneous observation of the entire mass spectrum.

The mass spectrometer used in the SPECTRO MS, has the potential to overcome all the problems associated with "scanning" spectrometers by allowing all the isotopes to be measured simultaneously. This is a double focusing magnetic sector field mass spectrometer in Mattauch-Herzog geometry (Fig. 2), which uses an ESA and a magnet, but in a specific geometric configuration that focuses ions of different mass / charge ratio onto the same flat focal plane at the exit of the magnet, but spatially separated. This is achieved without the need for varying either the voltages applied to the ESA or the strength of the magnetic field.
Unfortunately, however, until now there has been no commercially available detector capable of detecting and measuring this spatially resolved mass spectrum. Photographic plates have been used, but these have obvious limitations.
The SPECTRO MS solves all these problems by combining the Mattauch-Herzog geometry with a revolutionary new detector that measures the entire mass spectrum simultaneously.

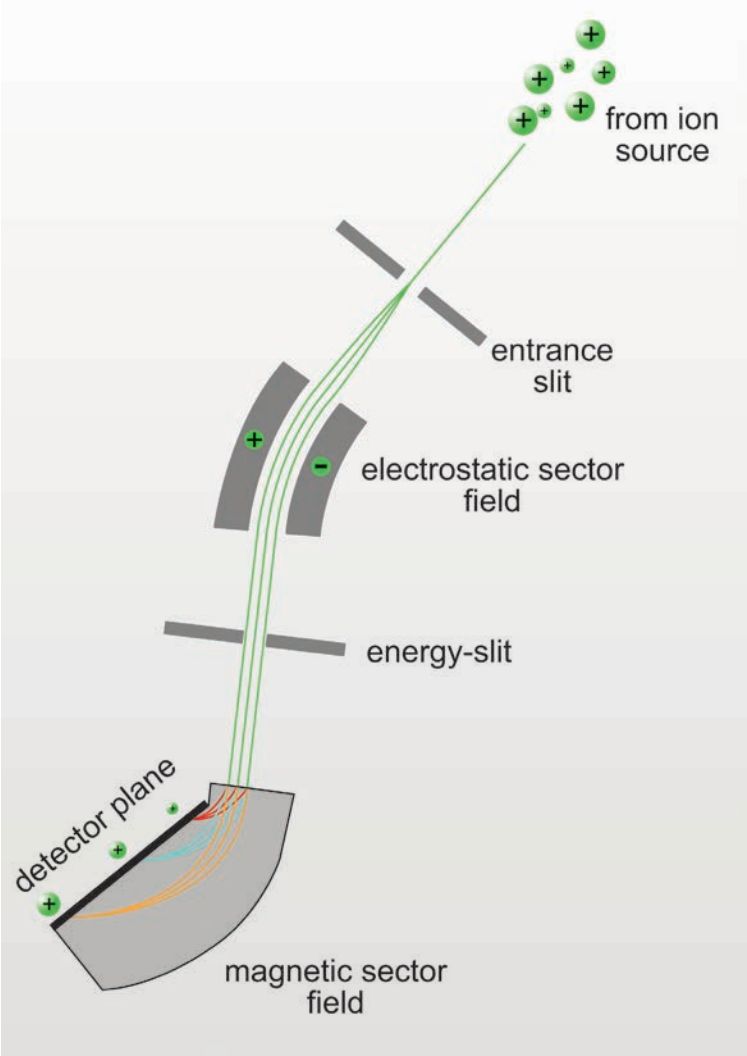

Figure 2: Double-focusing sector field mass spectrometer Mattauch Herzog geometry

The "Ion 120" Direct Charge Detector

Specially developed for SPECTRO, the Ion 120 is a 12 cm long CMOS (Complementary Metal Oxide Semiconductor) array that covers the entire mass range from ^{6}Li to ^{238}U in 4800 separate channels.
The basic principle of the detector is similar to that of a Faraday Cup: when a charged ion arrives at a detector element it is discharged by receiving an electron, generating a detector signal. The detector is referred to as a Direct Charge Detector (DCD) because every ion arriving at the detector contributes to the signal. The DCD can therefore be regarded as 100 % efficient. Furthermore, with 4800 detector elements covering the mass range (from ~5 to 240 amu) every mass unit is on average covered by 20 separate elements, resulting in a true mass spectrum rather than a single point for each atomic mass unit (amu).
Each detector channel incorporates separate high- and low-gain detector elements, allowing it to independently handle a wide range of signal levels. This in part accounts for the extremely

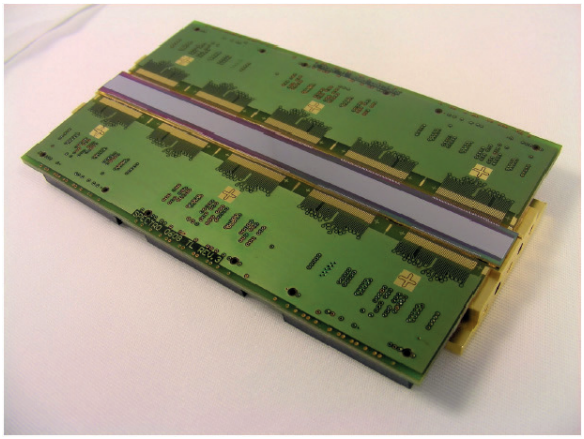

Figure 3: The "Ion 120" direct charge detector

wide dynamic range of the system, up to 8 orders of magnitude in the basic integration cycle. The dynamic range is extended even further by the readout system used when the measurement is longer than the basic integration cycle. In the basic integration cycle, each channel is monitored by the electronics at very short time intervals (every 20 ms). If the signal integrated in this time interval nears the threshold of the channel, the integrated signal of that channel is automatically logged and the channel is reset and begins to collect information again. This is repeated until the end of the set measurement time, when all the collected data is integrated to produce the final signal. This means that the detector is always working within its linear response range and that longer integration times can be used without fear of detector saturation.

Some conventional MS detectors, like the SEM (Secondary Electron Multiplier), and even the less sensitive Faraday Cup can show aging effects. SEMs are particularly prone to these aging effects and have to be replaced from time to time, making them an expensive consumable. No such aging effects have been observed with the Ion 120 detector.

Advantages gained by using this new detector may be summarized as:

- Elimination of noise from the sample introduction system
  - Flicker noise from the plasma
  - Pulsation from the peristaltic pump
- Improved quantitative precision theoretically by 1-2 orders of magnitude
- Element ratios (internal standards, isotope ratios) are calculated on readings obtained simultaneously and therefore under identical conditions
- Complete mass spectrum from every single measurement
- Method development can be conducted after the sample is measured to:
  - See unexpected interferences
  - Detect unexpected elements
  - Make interference corrections after the measurement is completed
  - Review spectra of samples that no longer exist
  - Determine additional elements
- Improved handling of transient signals
  - No signal skew when measuring many different isotopes
  - Full duty cycle on all elements independent of the number of elements monitored – all are measured, all the time.
  - Better precision with transient sample introduction techniques (HPLC, Laser Ablation, …)
- Improved productivity: the complete mass spectra of up to 100 samples per hour can be recorded with no limitations on the number of elements or isotopes measured.

THE SPECTRO MS

The benefits of simultaneous detection can only be fully realized if the rest of the instrument is functioning optimally, and the SPECTRO MS incorporates many other refinements:

Sample Introduction

The SPECTRO MS can be fitted with virtually any sample introduction system normally used in ICP-MS. The large sampling area can accommodate most types of nebulizer/spray chamber combinations, with a 12-roller, 4-channel, computer-controlled peristaltic pump fitted as standard. In addition to sample and drain, additional channels can be used for a variety of purposes such as online addition of internal standards.

The Plasma

Many pharmaceuticals are organic compounds, and organic solvents are very frequently used in organic synthesis. Many plasma systems become unstable when loaded with organic material, and in extreme cases the plasma may even be extinguished.

The energy needed to maintain the plasma is provided by a radio frequency generator. International regulations only allow certain frequencies to be used for this purpose, usually 27.12 MHz or 40.68 MHz. Limited external radiation is permitted at these frequencies whereas the use of other frequencies would require extensive shielding. A convenient way of coping with changing sample load is for the RF generator to adjust the resonant frequency so that the effective plasma power remains constant. This is termed a "free-running" generator. The generator used in the SPECTRO MS is an air-cooled free-running RF generator running at a nominal frequency of 27.12 MHz with a power output range from 0.7 to 1.7 kW. 27.12 MHz is chosen in preference to 40.68 MHz because the permitted bandwidth over which the frequency can be adjusted is wider at 27.12 MHz, giving more room for adjustment and thus better control (Fig. 4).

The generator used to power the plasma in the SPECTRO MS has a maximum output of 1700 W, more than enough for even the most difficult organic solutions.

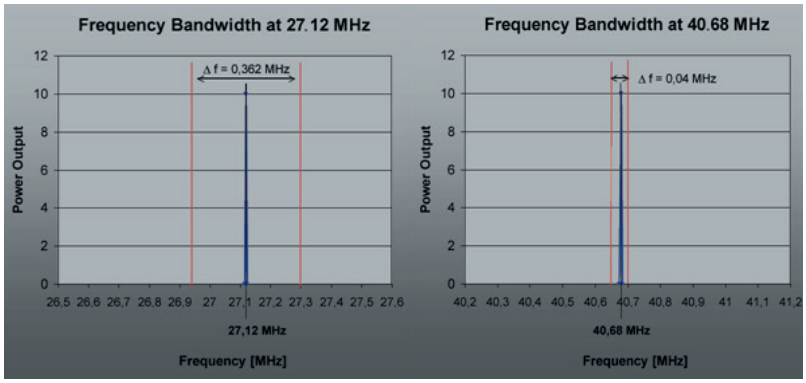

Figure 4: Permitted frequency bandwidths at 27.12 MHz and 40.68 Mhz

The Spectrometer Interface

The spectrometer interface provides the route between the plasma (at up to 10,000K and atmospheric pressure) and the mass spectrometer (at ambient temperature and high vacuum). The SPECTRO MS interface is an optimized two-stage design with "sampler" and "skimmer" cones. At the sampler cone, the pressure drop across the cone accelerates the ions to supersonic speed towards the skimmer cone in an expanding beam.

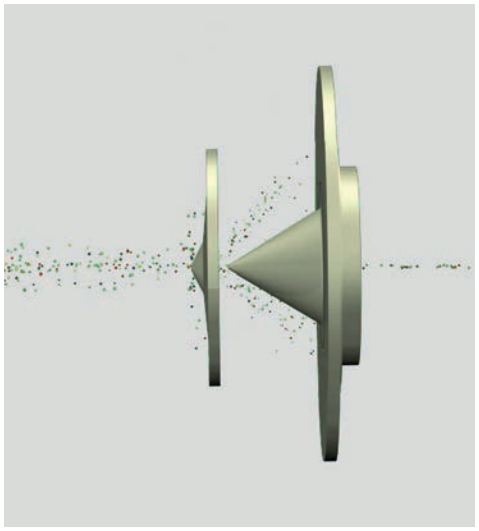

Figure 5: SPECTRO MS interface

At the skimmer cone the ions are further accelerated into the vacuum chamber of the spectrometer. Both cones "peel off" the outside of the plasma, removing most of the argon while maintaining the composition of the ions in the central channel. The cones are made of either nickel or platinum for aggressive acid and organic samples. Since these items can require regular inspection and cleaning, particularly with organic samples, the SPECTRO MS is configured such that a single mouse click brings the interface assembly into a convenient service position for maintenance without losing vacuum in the spectrometer.

Ion Optics

After passing through the interface, the stream of ions still contains unwanted particles such as neutral atoms, electrons, photons and dirt. The ion stream also needs to be shaped to conform to the entrance slit of the spectrometer.

In the SPECTRO MS this task is carried out by the components of the instrument's sophisticated ion optics:

1. The interface
2. Einzel lens, focuses the ion stream into
3. Pre filter, deflects the ions into a curved flight path, while neutral species, dirt and other particles fly straight on
4. Einzel lens, extracts the ions from the filter and focuses them onto the spectrometer entrance slit via
5. Quadrupole doublet, shapes the ion beam to optimally fill the spectrometer entrance slit.

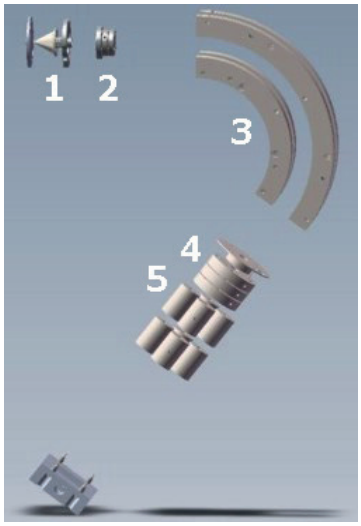

Figure 6: Ion optics

The Spectrometer

As noted above, the spectrometer in the SPECTRO MS is a double focusing sector field mass spectrometer in Mattauch-Herzog geometry, with an Electrostatic Analyzer (ESA) followed by a magnetic sector. The ESA separates ions based on their kinetic energy and the magnetic sector on the basis of their mass/charge ratio. All the ions are focused by the magnet onto the same focal plane, the location of the Ion 120 detector.

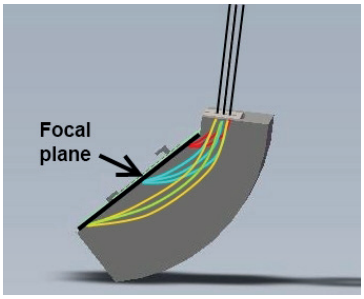

Figure 7: The magnet and detector

The complete mass spectrum is available for detection, without scanning: the ESA is at a fixed potential and the magnetic sector is a permanent magnet. This eliminates yet more potential variables from the system.

Result Processing and Software

The SPECTRO MS software is called MASS ANALYZER VISION and is based on SPECTRO's highly successful SMART ANALYZER VISION software used with their Optical Emission Spectrometers (OES), with special added functionality for processing mass spectra.

Compared to ICP-OES, the ICP-MS spectrum is simple, with the complete mass spectrum of the Periodic Table containing only approximately 220 masses of interest, compared to tens of thousands of emission lines for the optical emission spectrum. Every element is represented in the mass spectrum by one or more isotopes.

In spite of the relative simplicity of the mass spectra, spectral interferences between elements can still occur. Commonly encountered spectral interferences are:

- Isobaric interferences occur, when isotopes of different elements have identical mass/charge (m/z) ratios. Examples are Ba, Ce and La all of which have isotopes with mass/charge 138.
- Doubly charged interferences, which can occur with divalent ions such as $^{138}Ba^{++}$. This has a mass of 138, but being doubly charged has an effective m/z of 69 and would therefore interfere with $^{69}Ga^{+}$, also with an m/z of 69. These interferences occur mainly with elements having a low ionization potential, such as the alkaline earth and Rare Earth Elements (REE's).
- A third type of spectral interference can occur when polyatomic charged species such as BaO^{+} are formed in the plasma. This ion could interfere with the determination of Nd, Sm, Eu and Gd.

The interferences are well known and documented, and the SPECTRO MASS ANALYZER VISION software includes all the necessary data to make the appropriate corrections. Having the entire mass spectrum available makes this process much more reliable, to the extent that the SPECTRO MS has a "Semi-Quant" analysis mode that can use all this information combined with a "response table" containing sensitivities for all the elements, to give a good approximation of what an unknown sample contains even if no standards are available and the instrument has never been calibrated for some of the elements. Such a measurement takes only 10 seconds with the SPECTRO MS and can yield precision and accuracy in the range of 10-15 % or better.

The software also contains the resources to handle isotope ratio and isotope dilution calculations. In all isotope ratio studies, the fully simultaneous measurement of the complete mass spectrum improves accuracy and precision of the results by eliminating any noise from the sample introduction system and the plasma. For absolute concentration measurements, precision of 0.01% can be achieved using isotope dilution with the SPECTRO MS.

The software operates in the familiar Windows™ environment and is fully EPA, FDA, CLP and 21 CFR Part 11 compliant. The software also continuously monitors and documents the status of the instrument and uses SPECTRO's iCAL logic to ensure that the instrument is always in an optimum state of operation.

THE ANALYSIS OF PHARMACEUTICAL SAMPLES

Sample Preparation

Pharmaceutical products and components may be presented for analysis in many different forms – aqueous or organic solutions, suspensions, gels, powders, tablets and so on. As such, it is not realistic to propose a generic method of sample preparation. Furthermore individual materials may be covered by specific monographs indicating the sample preparation to be used. Forms of sample preparation include direct analysis, aqueous solution, organic solution, and digestion procedures, the latter used when a sample is not directly soluble in aqueous or organic solvents.

Digestion procedures are carried out in closed pressure vessels, often using a microwave oven to promote dissolution. This not only speeds up the dissolution process, but has the advantage that any volatile elements are contained and do not escape during the process.

An example procedure that has been shown to have broad applicability is given in chapter 233 of the guidance:

1. Dehydrate and predigest 0.5 g of primary sample in 5 ml of freshly prepared Strong Acid.
2. Allow to sit loosely covered for 30 min in a fume hood.
3. Add 10 ml more of strong acid, and digest, using a closed vessel technique, until digestion or extraction is complete.
4. Repeat if necessary by adding 5 ml more of strong acid. In this context "strong acid" is concentrated ultra-pure nitric, sulfuric, hydrochloric, or hydrofluoric acid or aqua regia.

The digested sample is then diluted as appropriate for measurement.

Samples and blanks may be spiked with target elements, i.e. those elements with the potential to be found in the sample. These always include lead, mercury, arsenic, and cadmium, and should also include any of the remaining elemental impurities listed in the tables above that may have been used in the manufacture of the product or its components.

One of the advantages of the inductively coupled plasma is that there is often sufficient energy to decompose and atomize solid particles that enter the plasma. In certain circumstance it is therefore possible to analyze samples such as suspensions or even slurries directly. Clearly in this case careful validation will be necessary to ensure that the physical nature of the sample has not compromised the result.

Performance and Results

One of the most important performance indicators for this type of instrumentation is the Limit of Detection (LOD), at which an analyte's presence can be detected (not quantified). ICP-MS is one of the most sensitive techniques available for elemental analysis, and LODs for the elements cited in the USP-NF are given in Table 4. For convenience, the table also shows the LVP limits for these elements. The Limit of Detection is the minimum concentration at which an analyte's presence can be detected: for accurate quantitative analysis, the minimum working level would normally be ten times the LOD. It can be seen that with the SPECTRO MS, the LOD is at least one thousand times lower than the specified levels.

Table 4: Limits of Detection in 0.09 % NaCl solution (Update February 2013)

Element	LVP Component Limit (µg/kg)	LOD (µg/L)
Inorganic arsenic	150	0.07
Cadmium	250	0.01
Lead	500	0.04
Inorganic mercury	150	0.007
Copper	10,000	0.05
Molybdenum	1,000	0.02
Nickel	5,000	0.07
Palladium	1,000	0.001
Platinum	1,000	0.004
Vanadium	1,000	0.05
Osmium	1,000	0.003
Rhodium	1,000	0.03
Ruthenium	1,000	0.03
Iridium	1,000	0.002

Note that these measurements were carried out in a 0.09 % saline solution. A potential problem with measuring traces of arsenic in saline solution is the relatively high level of chlorine present. This can introduce a potential interference due to the formation of argon chloride ArCl in the plasma, which has the same mass as arsenic (75) and could therefore add to the As signal. To investigate this effect on the SPECTRO MS, samples of 0.9 % saline infusion solution were diluted ten times, spiked with 10 ppb As and then measured using a standard addition calibration. Indium was used as internal standard. The results are given in Table 5.

Table 5: Measurement of As in high Cl matrix

	Spike conc. [µg/L]	Measured conc. [µg/L]	Recovery %
Replicate 1	10	10.08	101
Replicate 2	10	9.99	100
Replicate 3	10	9.85	98
Replicate 4	10	9.88	99
Replicate 5	10	9.85	99

No corrections of any kind were applied to these results, showing that the SPECTRO MS does not suffer from this interference at this Cl level. This facilitates the direct measurement of NaCl infusion solutions.

Most pharmaceutical samples are organic in nature, and as has been noted above, the presence of organic substances can have a destabilizing effect on the plasma. Many syntheses are base-catalyzed, but in organic media inorganic bases such as NaOH and KOH may not be suitable, and organic bases such as TMAH (Tetramethylammonium Hydroxide) are used instead. To test the effect of TMAH on the performance and stability of the SPECTRO MS, detection limits were measured in a 5 % TMAH (TraceSELECT Ultra, Fluka Sigma Aldrich) solution. The results are given in Table 6.

A four-hour stability run under these conditions using ^{115}In as internal standard shows the excellent stability that can be achieved with the SPECTRO MS, even with organic matrices:

It's sensitivity and stability make the SPECTRO MS ideal for the measurement of trace elements in pharmaceutical products, but it is also necessary to comply with the accuracy and reproducibility criteria of the USP-NF Chapter 233. The USP guidelines give a procedure for the validation of the analyses. This involves the measurement of defined standards and test samples under specified conditions to test the accuracy and repeatability of the method.

The section 'Alternate Procedure Validation' describes two different procedures for validating the analytical process: a limit procedure and a quantitative procedure. In this example, the quantitative procedure was used.

Table 6: Limits of Detection in 5 % TMAH

Element	LOD [µg/L]
V	0.07
Cr	0.2
Mn	0.03
Co	0.01
Ni	0.09
Cu	0.08
Zn	0.070
Ga	0.006
As	0.02
Se	0.7
Rb	0.007
Sr	0.006
Mo	0.02
Ru	0.02
Rh	0.005
Ag	0.02
Pd	0.01
Cd	0.01
Ba	0.006
Ir	0.005
Pt	0.03
Hg	0.005
Tl	0.007
Pb	0.009
Bi	0.004
U	0.004

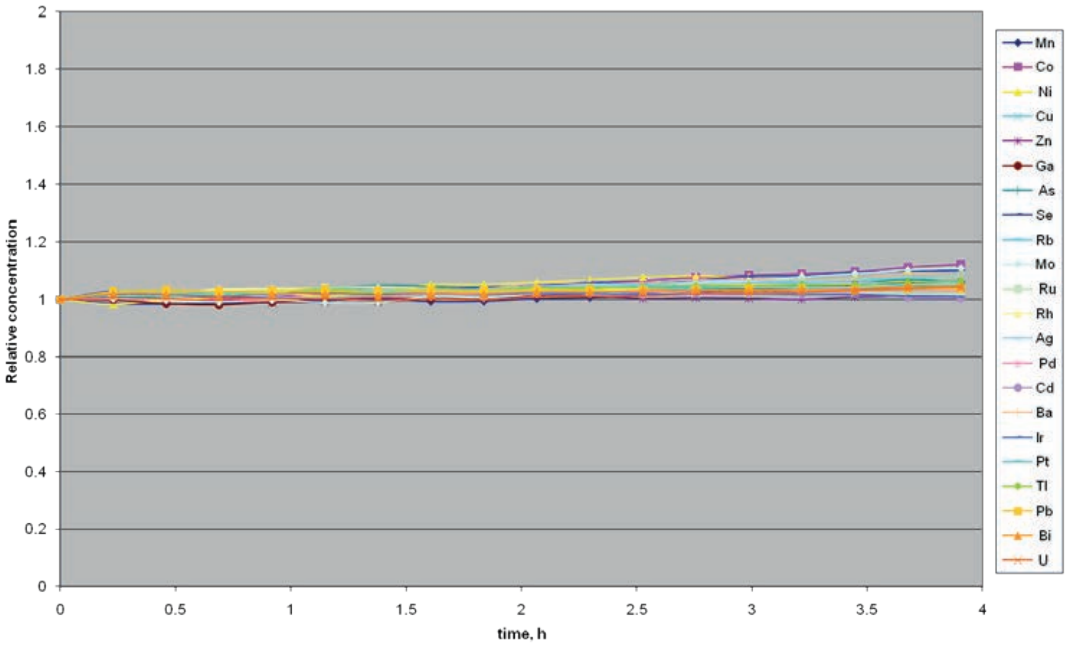

Figure 8: Stability of SPECTRO MS – 20 µg/l of the elements in 5 % TMAH solution

An over the counter (OTC) homeopathic medication in the form of tablets of 0.25 g each was used as sample. Two tablets each were dissolved in 5 ml conc. HNO_3 and 1 ml HCl under slight heating in an ultrasonic bath and filled up to 50 ml with DI water to a clear solution.
The target limit in [µg/g] in the material is calculated dividing the PDE by the maximum daily dose according to the prescription. A dose of 2 tablets every hour for 12 hours was the described maximum dose resulting in a maximum daily dose of 6g. The target concentration J is calculated by dividing the target limit by the dilution factor from the sample preparation, in this case 100. These numbers are shown in Table 7. A stock solution containing the elements a factor 100 above the target concentration was prepared from appropriate reference materials. This solution was used to set up the external standards and spike some of the solutions according to the procedures given for accuracy, repeatability and ruggedness.
The instrument was optimized according to the standard operating procedures using the Merck Multi Element Solution VI (Merck, Damstadt, Germany). An iCAL procedure and a DC measurement was performed prior to the analytical run. The instrument conditions used can be found in Table 8. Indium was used as internal standard for all target elements.

Table 7: Calculation of Target Limit, Target Concentration J, Standard and Spike concentrations

Oral Daily Dose PDE [µg/day]	Maximum daily dose of Tablet [g/day]	Target limit [µg/g]	Dilution with sample prep	Target concentration J [µg/L]	0.5 J	1 J	1.5 J	2 J
25	6	4.17	100	41.67	20.83	41.67	62.50	83.33
5	6	0.83	100	8.33	4.17	8.33	12.50	16.67
1.5	6	0.25	100	2.50	1.25	2.50	3.75	5.00
15	6	2.50	100	25.00	12.50	25.00	37.50	50.00
100	6	16.67	100	166.67	83.33	166.67	250.00	333.33
100	6	16.67	100	166.67	83.33	166.67	250.00	333.33
100	6	16.67	100	166.67	83.33	166.67	250.00	333.33
100	6	16.67	100	166.67	83.33	166.67	250.00	333.33
100	6	16.67	100	166.67	83.33	166.67	250.00	333.33
100	6	16.67	100	166.67	83.33	166.67	250.00	333.33
100	6	16.67	100	166.67	83.33	166.67	250.00	333.33
50	6	8.33	100	83.33	41.67	83.33	125.00	166.67
100	6	16.67	100	166.67	83.33	166.67	250.00	333.33
1000	6	166.67	100	1666.67	833.33	1666.67	2500.00	3333.33

Table 8: Measurement conditions

Plasma Power	1465 W
Coolant Flow	12 L/min
Neb Flow	0.90 L/min
Aux Flow	2.4 L/min
Nebulizer	concentric Sea Spray
Spray Chamber	cyclonic baffled, not cooled
Basic Integration time	20 ms
Replicate Time	20 sec
No. of replicates	3
Sample Flush	65 sec
Wash Time	50 sec

Table 9: Recovery of Standardization Solution 1 after sample measurements for drift control (Limit: 80 – 120 %)

Element	2J as sample [µg/L]	Expected concentration [µg/L]	Recovery [%]
Cd	85.3	83.33	102.4
Pb	16.3	16.67	97.8
Inorg As	5.06	5.00	101.2
Inorg Hg	49.9	50.00	99.8
Ir	323	333.33	96.9
Os	321	333.33	96.3
Pd	329	333.33	98.7
Pt	340	333.33	102.0
Rh	328	333.33	98.4
Ru	339	333.33	101.7
Mo	332	333.33	99.6
Ni	168	166.67	100.8
V	334	333.33	100.2
Cu	3302	3333.33	99.1

The calibration was performed using 3 matrix matched (acid and carbon) solutions: Blank, Standardization solution 1, containing 2 J, and Standardization Solution 2 containing 0.5 J. Standardization solution 2 was measured after the samples again to detect a potential drift. The criterion for drift for each target element is not to exceed 20%. The results are shown in Table 9.
From the prepared samples, 3 were measured directly to determine the concentrations for the elements according to the list in USP 232. The results for those elements including the limit of detection (LOD) under these measurement and lab conditions can be found in Table 10.

Table 10: Target Limits, LOD and results of 3 samples measured

Element	Oral Daily Dose PDE [µg/day]	Maximum daily dose of Tablet [g/day]	Target limit [µg/g]	LOD [µg/g]	Sample 1 [µg/g]	Sample 1 [µg/g]	Sample 1 [µg/g]
Cd	25	6	4.17	0.0003	0.010	0.007	0.008
Pb	5	6	0.83	0.0001	0.019	0.020	0.020
Inorg As	1.5	6	0.25	0.0005	0.039	0.047	0.048
Inorg Hg	15	6	2.50	0.0010	0.086	0.074	0.081
Ir	100	6	16.67	0.0001	0.013	0.010	0.007
Os	100	6	16.67	0.0007	< 0.0007	< 0.0007	< 0.0007
Pd	100	6	16.67	0.0009	< 0.0009	< 0.0009	< 0.0009
Pt	100	6	16.67	0.0003	0.020	0.008	0.004
Rh	100	6	16.67	0.0001	0.006	0.006	0.004
Ru	100	6	16.67	0.0008	0.024	0.019	0.017
Mo	100	6	16.67	0.0005	0.029	0.029	0.026
Ni	50	6	8.33	0.0010	0.038	0.042	0.036
V	100	6	16.67	0.0071	0.279	0.339	0.343
Cu	1000	6	166.67	0.0049	0.108	0.122	0.111

A part of the measured mass spectrum can be found in Figure 9. Looking through this mass spectrum, additional elements were be found and their presence in the sample detected by overlaying the isotopic pattern of these elements with the measured mass spectrum. In this way, the presence of the following elements could be detected in the sample in different concentrations: Na, Mg, Al, P, K, Ca, Cr, Mn, Fe, Zn, Se, Rb, Sr, Br, Zr, Nb, Sn, Sb, I, Ba, La, Ce, W and traces of U.
The suitability of the procedure has to be tested with additional tests. The accuracy of the procedure has to be tested by spiking three separate samples with the target elements at concentrations of 50 to 150% of J. The spike concentrations used in this test were 0.5 J, 1J and 1.5 J. A spike recovery of 70% to 150% has to be achieved for the mean of the three replicate preparations. The results presented in Figure 10. show a recovery for most elements and spike levels between 90 to 110 % and all well within the limits.
The repeatability has to be tested by spiking 6 independent samples of the material under test with the levels of 0.5J, 1J and 1.5J. The repeatability of those six measurements must be within 20% RSD for each target element. The results of these

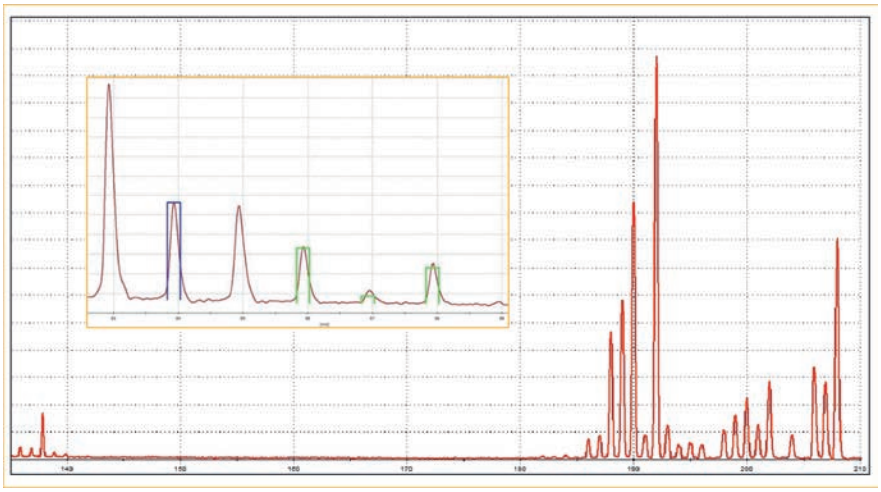

Figure 9: Mass Spectrum of Sample 1 with the insert showing the Zn isotope pattern

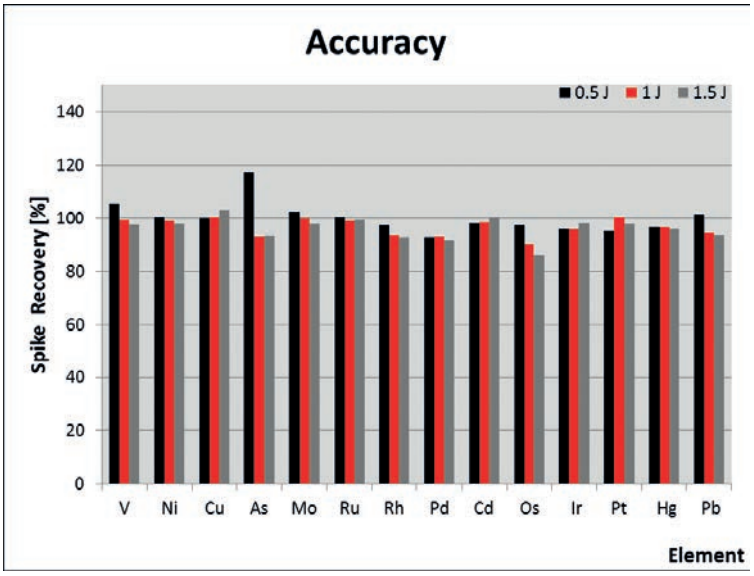

Figure 10: Spike Recoveries of 0.5J, 1J and 1.5J of three separate samples of material under test

measurements are shown in Figure 11. The RSD's achieved are within 2.5% for all target elements and spike levels.

The ruggedness has to be tested by repeating the repeatability test on different days or with different instrumentation or with different analysts. The test was repeated on different days. The criterion is a limit of not more than 25 % RSD for the results of each target element. The results presented in Figure 12 show RSD's of 6 % or better well within the defined limits.

It is stated in the description of the performance tests of the quantitative procedures, that the limits of quantitation, range and linearity are demonstrated by meeting the accuracy requirements. The LODs found in these measurements calculated out of 3 times the standard deviation of the measurement of the blank is about 3 orders of magnitude lower than the target concentration. This gives the opportunity to reduce the measurement time even further without losing the full spectrum availability and increasing sample throughput.

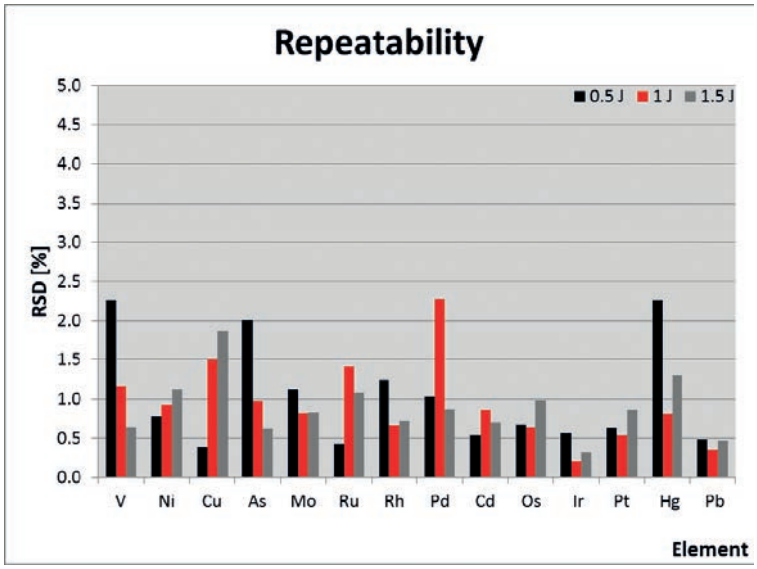

Figure 11: RSD's of six independent samples of material under test spiked with 0.5J, 1J and 1.5J

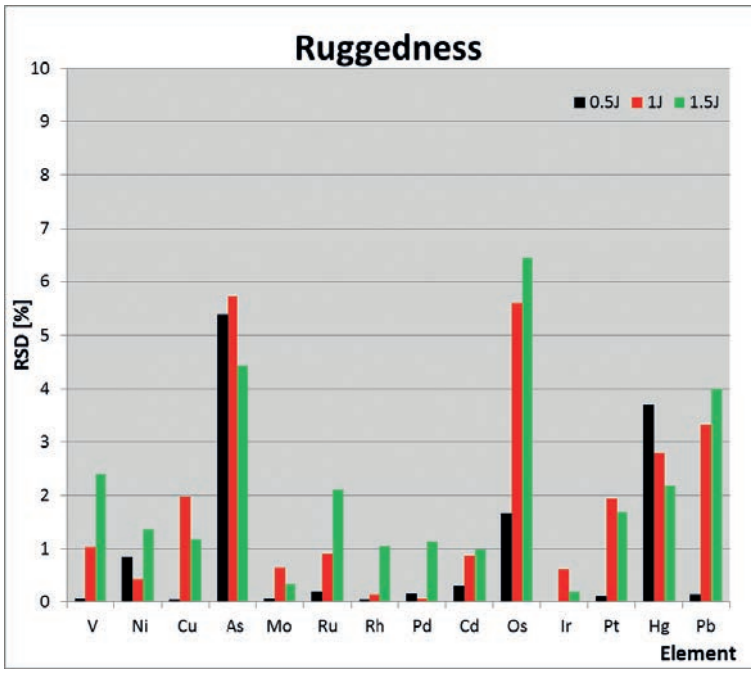

Figure 12: RSD of measurments performed on two different days

CONCLUSIONS

The SPECTRO MS is an innovative and powerful addition to the arsenal of the analytical chemist. It's unique ability to carry out truly simultaneous measurements on all elements from ^{6}Li to ^{236}U gives significant benefits in accuracy, repeatability and speed of analysis, attributes that make it especially suitable for the elemental analysis of pharmaceutical raw materials, intermediates and finished products. Additionally, the Mass Analyzer Vision software is 21 CFR part 11 compliant, including: data security, integrity and traceability. The full spectrum availability gives the complete information about the elemental impurities in every single sample measured. Post measurement reprocessing opens the possibility to add, eliminate or change corrections for polyatomic, isobaric or doubly charged interferences if required without having to analyze the sample again. Unexpected elements can be found and quantified to react on changes and differences in legislative procedures for the pharmaceutical industry. Even a retrospective evaluation of samples, that do not exist anymore, is possible.

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[1] Elemental Impurities, An industry perspective on harmonization and implementation of ICH and USP requirements, Pharmaceutical Technology, 11-2012, 58 - 64

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