Closed Vessel Digestion for Elemental Impurities Analysis of Osmium According to ICH Q3D, USP <232>, <233> and Ph. Eur. 5.20 with PDC

Using Multiwave 7000 simultaneously facilitates the digestion of different sample matrices under full GMP compliance and even generates digestion solutions enabling the accurate and reproducible determination of very difficult to determine Osmium.

1 Introduction

Since elemental impurities constitute not only a toxicological risk for the patient but also may affect the quality and efficacy of pharmaceutical products, their analysis plays an important role within the development and quality control of pharmaceuticals.

Due to new regulations in the United States Pharmacopoeia (USP <232> and <233>), the European Pharmacopoeia (Ph. Eur. 5.20) as well as the International Conference on Harmonization (ICH Q3D) the use of ICP-OES or ICP-MS together with reliable sample preparation techniques such as microwave-assisted closed vessel digestion has become state of the art for the quantification of elemental impurities.

Amongst other elements these regulations also define permitted daily exposure limits (PDE) for Osmium since the platinum element Os is known to be used as catalyst during the production chain of certain active pharmaceutical ingredients (APIs).

The digestion of the sample matrix mainly is done by oxidising mineral acids like HNO_3 which will cause problems in the determination of Os traces, since this element forms different species of varying volatility under such conditions which again leads to uncontrolled losses of Os. Osmium tetroxide e.g. is not only highly volatile but also very toxic by inhalation, ingestion and skin contact.

This report applies Multiwave 7000 and after the digestion a dilution with a stabilization solution containing acetic acid, ascorbic acid and thiourea in order to stabilize Os in the oxidizing environment during the sample preparation of one pharmaceutical product and one API.

2 Instrumentation

The digestion was performed in Multiwave 7000 together with Rack 18 with 18 mL pressure-sealed quartz vials and 15 mL sealed quartz vessels.

The quantitative analysis was performed on ICP-MS, Agilent 7900.

Figure 1: Multiwave 7000

Figure 2: 18 mL Pressure-Sealed Quartz Vials and 15 mL Sealed Quartz Vessels in Rack 18

3 Experimental

The samples were spiked with Os at 100 % of the J level (target concentration after sample preparation). The performance of the digestion was evaluated by calculating the recovery rates of the measured spiked samples in relation to the theoretical values (unspiked sample + spiked value). All spiked samples were prepared in triplicate (n=3).

3.1 Samples

Cold Syrup

Containing a triple active ingredient complex and high amounts of alcohol, sugar and glycerin

Nicotinic Acid, API

Aromatic API requiring temperatures above 200°C for total

3.2 Preparation of Sample Solutions

For both products the limits for the oral dosage form stated in USP<232> were considered:

Oral PDE: 100 µg/day

Dividing the PDE Limit by the respective maximum daily dose of each product the target values in [µg/g] were calculated:

	Cold Syrup	API, Nicotinic Acid
Maximum Daily Dose According to the Leaflet	30 mL	10 g *)
Approx. Weight per Dosage Unit [mg]	1120 **)	-
Approx. Absolute Maximum Daily Dose [g]	34	10
Target Limit [µg/g]	2.98	10.0

Table 1: Target Limits

**) Finished product containing Nicotinic Acid as API is considered to be dosed at a maximum daily dose of 10 g/day.*

***) Approx. weight per mL*

The **J level** considers also the sample weight of each product and the dilution after the digestion and thus is equal to the concentration of Os after the sample preparation step. In the following table the concentrations are presented at the stage before the final dilution of the digested solution for the measurement with the ICP.

	Cold Syrup	API, Nicotinic Acid
Sample Weight	1 mL approx.1120 mg	500 mg
J Level [µg/L]	3.34	5.00

Table 2: J Levels after dilution of 400 µL Digestion Solution in 20 mL of Stabilization Solution

Respective amounts of a 10 mg/L Os solution were gravimetrically spiked to the weighed sample before the digestion run in order to achieve the above mentioned J levels. Each spike solution was prepared in 2 % HCl by gravimetrical dilution of a 1000 mg/L stock solution.

The following amounts of reagents were used:

Reagent	Cold Syrup	API, Nicotinic Acid
H_2O *)	1.4	1.4
HNO_3	3	5
HCl **)	1	1

Table 3: Used Amounts of Reagents

**) The volume for H_2O is meant for the unspiked sample. For the spiked samples a corresponding volume of water was added to reach the same volume in total (spike volume and added volume of water = 1.4 mL).*

***) HCl was added after digestion.*

To keep potential losses of Os as low as possible especially for samples which show some reactivity already at room temperature it is recommended to perform the individual steps of the sample preparation procedure as fast as possible and in the following order:

- Weighing of the sample
- Addition of water
- Addition of spike solution
- Addition of HNO_3
- Closing the vial (with plug-on cap) and vessel (with PTFE strip and quartz lid)
- Performing the digestion according to 3.3
- Addition of HCl after the digestion
- Dilution with stabilization solution

Since the cold syrup also tends to vigorously exothermically react with HNO_3 the sealed quartz vessels were continuously cooled. Cooling was performed with cold water beginning from the addition of acid till the digestion run was started, only interrupted by the sealing procedure. Additionally the individual steps from weighing till closing for the sealed quartz vessels were subsequently performed for each vessel to keep the time where Os might be lost due to “open conditions” as short as possible. By this means it was possible to totally suppress the reaction for the cold syrup under room temperature in the sealed quartz vessels.

For the pressure-sealed vials the cold syrup only was cooled with water once the exothermic reaction started till it calmed down.

After each vial and vessel was closed they were put into Rack 18. The pressure-sealed quartz vials and sealed quartz vessels were processed in two different runs although it would be possible to run them together in one rack if there were less than 18 vials or vessels. The rack was put into the liner already filled with load solution (150 mL of water and 5 mL of HNO_3). The liner was put into the Pressurized Digestion Cavity (PDC) and the digestion program was started.

After the run 1 mL of HCl was added. Subsequently the samples were transferred into 50 mL tubes and filled up to 20 mL. 400 µL of this solution were diluted into 20 mL of the following aqueous stabilization solution in a separate 50 mL tube.

Acetic Acid	Thiourea	Ascorbic Acid
0.5 % (5 mL/L)	0.01 mol/L (761.2 mg/L)	0.1 g/L

Table 4: Stabilization Solution[1]

These sample solutions and all standard solutions were diluted with the stabilization solution to a final concentration of Os below 1 µg/L (dilution factor for the sample solutions: 1:10). All solutions were analyzed on the ICP-MS using Hf, Lu and Re (final concentration 10 µg/L) as internal standards.

3.3 Temperature Program

Starting pressure: 65 bar
Max. Pressure: 160 bar
Cooling temperature: 50°C
Pressure release rate: 10 bar/min

Step	Time [min]	Temperature [°C]
1	35	250
2	10	250

Table 5: Temperature program

4 Results

The measured recoveries as well as the relative standard deviations lie well within the respective limits stated under procedure validation in USP <233>. The LOQ in the measuring solution on the ICP was determined to be 0.05 µg/L.

The sealed quartz vessels are hermetically closed and therefore protected against potential losses of Os as soon as the sealing procedure (closing with PTFE strip and quartz lid and wrapping with PTFE strip) was finished. On the opposite the pressure-sealed quartz vials only are securely closed when the starting pressure was reached. Thus the time where the sample container is open can be reduced with use of the sealed quartz vessels which comprehensibly favors higher recovery rates.

Individually optimized handling steps like e.g. rapid handling or cooling generally improve the recovery rates also for demanding samples like carbonate containing matrices where the addition of acid triggers the formation and outgassing of carbon dioxide which again could be a reason for significant losses of Os. Therefore there also might be still some room to improve the recoveries of the cold syrup in the pressure-sealed vials since there the cooling with water before applying the vials to the instrument was not performed in a comparable optimized way to the sealed quartz vessels.

Pre-tests demonstrated that for pressure-sealed quartz vials the recovery rates are decreasing applying starting pressures below 65 bars. That is how the recovery rates increases in the range of approximately 30 % between a starting pressure of 35 and 65 bars. Additionally it was observed that the measurement on the ICP should be performed soon after the stabilization step since re-measurements of samples around 18 hours after the stabilization step demonstrated a decrease of the recovery up to 9 %. Solutions which were not diluted with the stabilization solution after the digestion lead to quite overestimated recovery rates up to 200 % and more

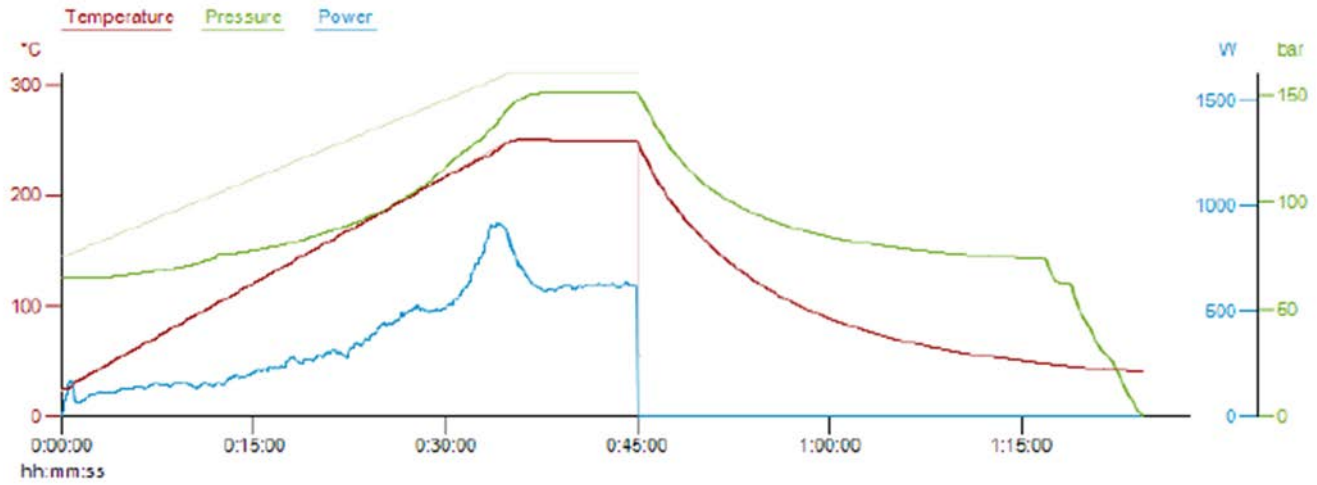

Figure 3: Run data, Sealed Quartz Vessels

		Cold Syrup	API, Nicotinic Acid	USP <233> Limits
Unspiked Sample [µg/g]		< 0.0456 (LOQ)	0.122	-
Spiked Sample	Measured Value [µg/g]	2.10	7.99	-
	Theoretical Value	2.89	9.76	-
	Recovery [%]	**72.9**	**81.9**	**70 - 150**
	RSD [%]	**4.4**	**3.0**	**≤ 20**

Table 6: Recovery Rates and Relative Standard Deviations, Pressure Sealed Quartz Vials

		Cold Syrup	API, Nicotinic Acid	USP <233> Limits
Unspiked Sample [µg/g]		< 0.0456 (LOQ)	0.122	-
Spiked Sample	Measured Value [µg/g]	2.42	8.91	-
	Theoretical Value	2.90	9.84	-
	Recovery [%]	**83.3**	**90.6**	**70 - 150**
	RSD [%]	**6.0**	**3.5**	**≤ 20**

Table 7: Recovery Rates and Relative Standard Deviations, Sealed Quartz Vessels

5 Conclusion

The suitability of Multiwave 7000 combined with ICP-MS was successfully verified for the quantitative determination of Osmium on a pharmaceutical product (cold syrup) and nicotinic acid as representative of an API.

The application of pressure-sealed quartz vials and the dilution with a stabilization solution containing acetic acid, ascorbic acid and thiourea after the digestion yielded to average recovery rates of 77 % and RSDs of max. 4 %. The use of sealed quartz vessels and optimized handling steps lead to higher recovery rates - on average 87 % - and a maximum RSD of 6 %. All values lie well between the limits stated in USP <233> (70 – 150 % for the recovery and not more than 20 % for the RSD).

With Multiwave 7000 Anton Paar offers a reliable, powerful and fully GMP compliant system for sample preparation for the determination of elemental impurities according to all current regulatory requirements (ICH, USP, Ph. Eur.). The instrument provides a FDA 21CFR Part 11 conform software and a meaningful pharmaceutical qualification documentation according to USP <1058> and GAMP 5.

6 References

[1] Cornel Venzago et al., J. Anal. At. Spectrom., 2013, **28**, 1125

Contact Anton Paar GmbH
Tel: +43 316 257-0
asc@anton-paar.com | www.anton-paar.com