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Development of analytical methods for the determination of sub-ppm concentrations of palladium and iron in methotrexate

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Abstract

Analytical methods for limit test (1 $\mu g\,g^{-1}$) determination of iron and palladium in the drug substance methotrexate (MTX) were developed. The methods developed were based on microwave-assisted, vapor-phase digestion using quartz inserts inside the digestion vessels, followed by instrumental determination. Iron was determined by graphite furnace atomic absorption spectrometry (GFAAS) and palladium by direct current plasma optical emission spectrometry (DCP-OES). Detection limits of 0.20 $\mu g\,g^{-1}$ for iron by GFAAS and 0.30 $\mu g\,g^{-1}$ for palladium by DCP-OES in MTX were obtained. The validity of the methods was studied by spike recovery tests and by analyzing certified reference material (NIST 8433 corn bran, Fe determination) and an organometallic compound ($[(C_6H_5)_3P]_2PdCl_2$, Pd determination). In addition, the specificity of the GFAAS technique for iron determination was confirmed by comparing the results obtained by GFAAS with those obtained by hexapole collision cell, inductively coupled plasma mass spectrometry (ICP-MS).

Keywords: Methotrexate; Vapor-phase microwave digestion; Palladium; Iron; Graphite furnace atomic absorption spectrometry (GFAAS); Direct current plasma optical emission spectrometry (DCP-OES); Inductively coupled plasma mass spectrometry (ICP-MS)

1. Introduction

Quality control is an essential part of the manufacturing process of pharmaceutical products. The determination of potential impurities in different stages of the manufacturing process, and especially in the final product, is therefore necessary. Palladium and its compounds are widely utilized as catalysts in the synthesis of pharmaceutical products. It is therefore also one of the potential impurities in the final products and is routinely monitored. Other potential impurities, including iron, are associated with the manufacturing processes of pharmaceuticals. The concentration limits for these impurity elements in pharmaceuticals are usually defined by the supervising authority and/or by the customer of pharmaceutical bulk products. Control

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of these possible impurities is usually performed using so-called limit tests. Thus, reliable analytical methods are needed for the determination of impurities in pharmaceuticals.

Sample preparation is usually a critical step in pharmaceutical limit tests because of the relatively low detection limits ($<1\ \mu g\ g^{-1}$) and difficult (sometimes harmful) sample matrices. In some cases the samples can be dissolved in suitable acids, solvents or solvent mixtures before, e.g. palladium, determination [1,2]. On the other hand, when the sample matrix is difficult to dissolve/digest, microwave-assisted digestion methods using different acid mixtures should also be considered for the analysis of impurities. Contamination is also a crucial factor related to sample preparation, especially when low concentrations of certain naturally abundant analytes (e.g. iron) are being determined. One advantage of microwave-assisted digestion methods is the low risk of contamination. In addition, the use of vapor-phase microwave digestion makes it possible to further reduce contamination and also to achieve better digestion efficiency [3,4].

Different spectrometric techniques like graphite furnace atomic absorption spectrometry (GFAAS), optical emission spectrometry (OES) based on argon plasmas, and inductively coupled plasma mass spectrometry (ICP-MS), are widely used for the determination of analytes in environmental, clinical and industrial samples [5–8]. Some GFAAS and ICP-MS methods for the determination of iron [9] and palladium [1,2,10–12] in pharmaceutical products have been reported. The detection limits of these techniques are usually low enough for the determination of iron and palladium as limit tests in pharmaceutical samples. In addition, ICP-MS offers a multi-element capability and the detection limits obtained by ICP-MS are usually several magnitudes lower than those obtained by GFAAS. On the other hand, the use of organic solvents [13] or high amount of total dissolved solids (TDS) may cause problems in ICP-MS determinations and, in some cases, compromises have to be made when choosing a suitable analytical technique.

The aim of this work was to develop and validate methods for the determination of iron and palladium impurities in methotrexate (MTX) as a limit test ($1\ \mu g\ g^{-1}$). The methods developed were based on microwave-assisted, vapor-phase digestion using quartz inserts inside the digestion vessels, and the determination of iron by GFAAS and palladium by direct current plasma optical emission spectrometry (DCP-OES). The determination of low concentrations of iron is difficult in routine laboratories due to the high risk of contamination. Thus, vapor-phase microwave digestion offers a clear advantage over the other microwave oven digestion methods. The validation characteristics in limit tests that require clarification are the specificity and detection limit of the method [14]. Because sample digestion is a crucial part of the procedure owing to problems in digesting the sample matrix (MTX), the reproducibility and efficiency of the digestion should also be investigated. Validation of the developed methods was performed by determining the detection and quantitation limits for palladium and iron, by studying spike recoveries, and by analyzing reference material. In addition, the specificity of the GFAAS technique for iron determination was studied by comparing the results obtained by GFAAS with those obtained by hexapole collision cell ICP-MS.

2. Materials and methods

2.1. Instrumentation

A SpectraSpan IIIB DCP-OES (SpectraMetrics, Inc.) instrument was used in the determination of palladium. The instrumental parameters used for DCP-OES were as follows: wavelength 340.458 nm, input slit 100 μm × 300 μm, exit slit 100 μm × 300 μm, PMT voltage 650 V, nebulizer pressure 1.5 bar, sleeve pressure 3.5 bar and integration time 3 × 5 s. Background correction was not necessary due to the simple matrix of the digested samples.

A Perkin Elmer Zeeman/3030 atomic absorption spectrometer, equipped with a Zeeman effect background correction system, a HGA-600 graphite furnace and an AS-60 autosampler, was used in the iron determinations. A hollow cathode lamp (HCL) was used as the light source (operating current 30 mA). All the measurements were based on integrated absorbance. The wavelength used was 248.3 nm (slit 0.2 nm). Pyrocoated graphite tubes, with integrated platforms, were used for the atomization of iron (10 μl sample volume). The optimized graphite furnace program is given in Table 1. In addition, a Thermo El-

Table 1
The GFAAS temperature program for iron determination

Parameter	Dry			Ash	Atomization	Clean up	Cool
Set temperature (°C)	120	200	300	1000	2300	2650	20
Ramp (s)	1	20	20	20	0	1	1
Time (s)	15	10	10	10	5	5	5
Read					On		
Argon (ml min^{-1})	300	300	300	300	0	300	300

emental X7 ICP-MS (Thermo Elemental, Winsford, England), equipped with collision cell technology (CCT), was also used in the iron determinations. The ion lens settings, nebulizer gas flow rate and torch position of the instrument were optimized to obtain the maximum (>60,000 counts s^{-1} μg^{-1} l^{-1}) ^{115}In count rate. Iron was determined using the major iron isotope ^{56}Fe. Premixed 7% H_2 in He gas (5 ml min^{-1}) was used in the collision cell. More information about the optimization of collision cell gas flows and comparison of different collision gases against $^{40}Ar^{16}O$ is presented in our previous study [15].

An ASTRO 2001 System 2 TOC analyzer, equipped with an auto-sampler, was used in the total organic carbon (TOC) determinations on digested MTX samples. In this analyzer, $K_2S_2O_8$/UV radiation is used to oxidize organically bound carbon to CO_2. The released CO_2 is measured by an IR detector.

2.2. Reagents and gases

The ultrapure water used in this work was prepared with an ELGA UHG water purification system. Commercial stock solutions (1000 mg l^{-1}) of Fe and Pd were obtained from Merck. A multi-element tuning solution (Accutrace, ICP-MS tuning solution, AccuStandard, Inc.) was used in ICP-MS optimization. The other reagents used were as follows: potassium hydrogen phthalate ($KH(C_8H_4O_4)$, Fisher, p.a.), nitric acid (65% HNO_3, Merck, supra pur), hydrochloric acid (30% HCl, Merck, supra pur) sulfuric acid (95–97% H_2SO_4, J.T. Baker, p.a.), $[(C_6H_5)_3P]_2PdCl_2$ (98%, Aldrich). MTX samples were obtained from Fermion (Orion Corporation, Finland). NIST SRM 8433 (corn bran) was used for method performance evaluation. Argon and the collision gas (7% H_2 in He, H_2, 99.9 and He 99.996) were obtained from Messer.

2.3. Sample digestion method

The samples were digested in an MLS-1200 microwave oven (Milestone Corporation, maximum power 1025 W). Teflon® PFA Advanced Composite Vessels (CEM Corporation, 100 ml, maximum pressure 200 psi and maximum temperature 200 °C) equipped with Milestone QS-50 quartz inserts, were used in all sample digestions.

Before digestion, the quartz inserts were cleaned with aqua regia (4 ml) by heating them inside a microwave vessel for 15 min at 170 W (12 vessels). The inserts were then rinsed several times with ultra pure water.

The microwave sample digestion procedure used was modified from the methods developed in earlier studies [3,4]. In this procedure, 200 mg of MTX sample was weighed carefully into the quartz insert. The insert was then lowered into the bottom of the digestion vessel where a glass holder kept the insert approximately 2.5 cm above the bottom of the microwave vessel. One milliliter of concentrated H_2SO_4 and 0.5 ml of

Table 2
Digestion program (12 samples)

Stage	Reagents		Power setting[a]
	In sample	Outer	
1	0.5 ml HNO_3 1 ml H_2SO_4	3.5 ml HNO_3	5 min, 256 W 5 min, 294 W 20 min, 333 W 10 min, 380 W
2	None	None	5 min, 294 W 5 min, 333 W 20 min, 380 W 10 min, 333 W

[a] Power setting for 12 vessels. The vessels were cooled to room temperature and vented between the first and second stage.

concentrated HNO_3 were added directly onto the sample. Three milliliters of concentrated HNO_3 was added outside the quartz insert. The vessels were closed and 12 samples were heated at the same time in the microwave oven using microwave program 1 described in Table 2. After program 1 had been completed, the vessels were cooled down to room temperature, vented carefully, and the digestion continued using heating program 2 (Table 2). After the digestion the samples were diluted directly in the inserts to 10 or 20 ml with ultrapure water. The DCP-OES and GFAAS measurements were made directly on these solutions. For ICP-MS determinations all the samples (20 ml) were diluted 1:10 with ultrapure water. The acid concentration of the standards used in the DCP-OES and the ICP-MS determinations were matched to the estimated acid concentration in the final sample solutions. In the GFAAS measurements the standards were prepared in 2% (v/v) HNO_3.

3. Results and discussion

3.1. Detection and quantitation limits for iron and palladium

The detection limits of the methods were determined by measuring blank samples that passed through the whole procedure (in different batches) including microwave digestion. The limits of detection (LOD) and quantitation (LOQ) were defined using the following equation:

$$\text{LOD (or LOQ)} = c_b + ks_b \quad (1)$$

In this equation, c_b is the average blank concentration and s_b is the standard deviation of the replicate blanks. A value of 3 was used as coefficient k for calculating the detection limit, and a value of 6 for the quantitation limit. The detection limits and quantitation limits obtained for palladium and iron are shown in Table 3.

Table 3
The detection and quantitation limits for palladium and iron

Element	Instrument	n	Detection limit ($\mu g\,g^{-1}$)[a]	Quantitation limit ($\mu g\,g^{-1}$)[a]
Fe	GFAAS	14	0.20	0.41
Pd	DCP-OES	18	0.30	0.60

[a] Expressed as equivalent concentration in 200 mg sample.

The detection and quantitation limits obtained for iron and palladium were clearly below the required limit ($1\ \mu g\,g^{-1}$) defined for MTX. It is worth noting that it is easier to state that a certain concentration of analyte is present in the sample (decision limit) than that a certain concentration is not present in the sample (detection limit) for the given confidence level. Thus, the detection limit should be lower than or equal to the required limit test value. If quantitative results are required, then the quantitation limit should be lower than the limit value.

3.2. Specificity of the selected analytical methods

When the specificity of atomic spectrometric methods is evaluated, both matrix effects and spectral interferences must be studied. Of the different statistical techniques available, regression analysis is the most suitable [16]. This technique can be used in a number of different ways, as discussed in the following.

The existence of matrix effects in palladium determination by DCP-OES was investigated by comparing the slopes of the regression lines obtained by external calibration and the method of standard additions. The slopes of the regression lines, as well as their 95% confidence intervals, are shown in Table 4. Standard additions to the MTX samples (0, 0.02, 0.05, $0.100\ mg\,l^{-1}$) were made before microwave digestion. The slopes of the lines do not differ at the 95% confidence interval, and no spectral interferences were observed. Thus, the determination of palladium in digested MTX samples by DCP-OES is a highly specific method.

The specificity of the iron measurement was checked by comparing the results obtained for digested MTX solutions with GFAAS to those obtained by hexapole collision cell ICP-MS. Comparison of the GFAAS and ICP-MS methods was made by means of regression analysis. Fig. 1 shows the results obtained by the GFAAS and ICP-MS methods. The target values of the intercept (=0) and the slope (=1) are within the confidence intervals ($P < 0.05$) calculated for the regression line (intercept $= 0.0019 \pm 0.0127$, slope $= 1.115 \pm 0.164$). This is a clear indication that there are no systematic differences between the GFAAS or ICP-MS techniques when iron is determined in MTX. This is also a strong indication that both techniques are free from matrix effects and

Table 4
The slopes of the regression lines obtained by external calibration and the method of standard additions for palladium

External calibration[a]	Coefficient	*P*-value	Lower 95%	Upper 95%
Slope	19235	1.46E−07	18988	19483
Method of standard additions[b]				
Slope	18244	0.00145	15232	21257

[a] $n = 5$, replicate measurement on all five standards.
[b] $n = 3$, replicate measurement on all four standard addition samples.

spectral interferences. On the other hand, the results also show that it is possible, by employing a collision cell before the quadrupole mass analyzer in ICP-MS, to determine iron in MTX samples using the major iron isotope (^{56}Fe). The use of a collision cell is necessary because the polyatomic interferences, due to the argon-based molecule ($^{40}Ar^{16}O$), cause spectral overlap of the major iron isotope [17,18].

3.3. Recovery tests and efficiency of the digestion method

The recoveries of palladium were studied by adding small volumes (20–100 μl) of a palladium standard solution to the MTX samples ($n = 3$, spiked concentrations 0.02–0.100 mg l^{-1}). The samples were decomposed in a microwave oven and the recoveries for palladium were calculated after DCP-OES measurement. The recoveries of spiked samples ranged from 92 to 102%, which was an acceptable result. The recoveries of iron were studied by adding known amounts of an iron standard solution to the sample matrix. Both undigested samples ($n = 22$, spike 0.02 mg l^{-1}) and digested sample solutions ($n = 26$, spike 0.01 mg l^{-1}) were spiked. The average recoveries measured by GFAAS were 94.3% for samples spiked before digestion and 98.8% for samples spiked after digestion.

The recovery of palladium and iron, as well as the effiency of the digestion method, was also studied by digesting reference materials. To the best of our knowledge, however, there are no suitable pharmaceutical reference materials available for palladium or iron determination. As a result, an organometallic compound

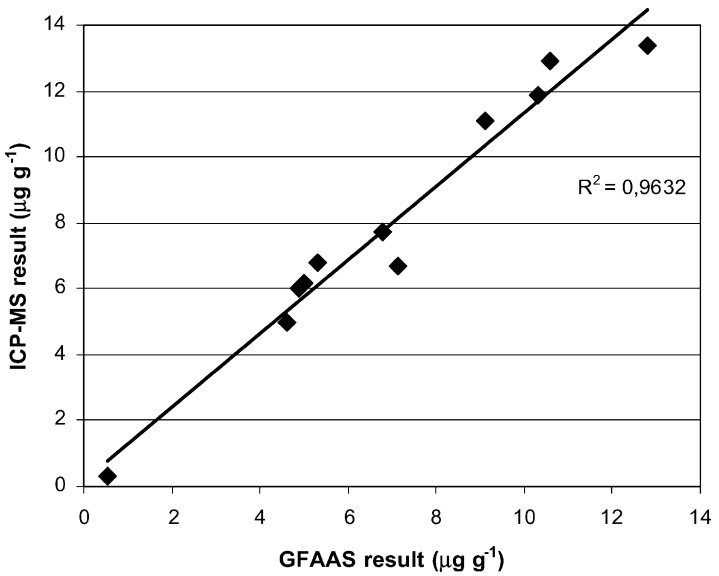

Fig. 1. Comparison of the GFAAS and ICP-MS methods using a linear regression model. ICP-MS data for the ^{56}Fe isotope.

Table 5
The amounts of added palladium (as $[(C_6H_5)_3P]_2PdCl_2$), measured palladium and recoveries for palladium with DCP-OES

Pd (mg)		
Added	Measured	Recovery (%)
0.079	0.072	91.3
0.064	0.061	95.2
0.030	0.029	95.5
0.048	0.044	92.5
0.025	0.023	92.8
0.0096	0.0101	105.6

($[(C_6H_5)_3P]_2PdCl_2$) was used for palladium and NIST 8433 (corn bran) standard reference material for iron.

0.063–0.532 mg of palladium compound was weighed on a microbalance into the quartz insert. In addition, 200 mg of MTX sample was also weighed into all the quartz inserts as a matrix compound. The samples were then digested in the microwave oven and the palladium concentrations measured by DCP-OES. The amounts of added and measured palladium, as well as their recoveries, are shown in Table 5. The average recovery of palladium was 95.5%.

The NIST 8433 (corn bran) standard reference material was also digested in a microwave oven. The iron results obtained by GFAAS are shown in Table 6. The average recovery of iron from the material was 101.1%. Thus, the good recoveries in the spiking experiments and from the digested reference materials show that iron and palladium can be completely recovered by the method based on microwave-assisted digestion and DCP-OES or GFAAS determination.

The residual carbon content of the digested samples was calculated using the results of the TOC analysis. The digestion efficiency of the 200 mg MTX sample was $99.7 \pm 0.3\%$ (mean $\pm$ s, $n = 9$). The efficiency of the digestion method is therefore excellent, and this digestion method can be used for MTX samples. In addition, the results from earlier oxidation efficiency measurements with different sample materials and almost the similar method [3,4] were in good agreement with these values.

Table 6
Iron recoveries measured for standard reference material NIST 8433 corn bran

Element	Instrument	n	Certified (mg kg^{-1})	Measured[a] (mg kg^{-1})
Fe	GFAAS	7	14.8 ± 1.8	15.0 ± 2.8

[a] Mean ± standard deviation.

4. Conclusions

The methods developed for palladium and iron can be used for a limit test of the drug substance methotrexate. The detection limits obtained (0.20 μg g^{-1} for Fe and 0.30 μg g^{-1} Pd) were clearly under the defined limit (1 μg g^{-1}). According to the results, there were no matrix effects in either the determination of palladium in digested MTX samples by DCP-OES or in the determination of iron in MTX by GFAAS. In addition, the good results obtained in the spike recovery tests, analysis of reference materials ($[(C_6H_5)_3P]_2PdCl_2$ for palladium and NIST 8433 corn bran for iron) and TOC analysis suggest that the digestion method is suitable for MTX digestion.

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