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Development and validation of a general non-digestive method for the determination of palladium in bulk pharmaceutical chemicals and their synthetic intermediates by graphite furnace atomic absorption spectroscopy

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Abstract

A simple, selective, sensitive, accurate and relatively inexpensive method for the determination of palladium in bulk pharmaceutical chemicals (BPC) and their synthetic intermediates by graphite furnace atomic absorption spectroscopy has been developed and validated. Sample preparation by direct dissolution of sample in 70% nitric acid is simple and effective without adverse effects. The limit of detection and the limit of quantitation of the method were determined to be 0.7 ppm and 2 ppm respectively in BPC.

Keywords: Bulk pharmaceutical chemicals; Graphite furnace atomic absorption spectroscopy; Palladium

1. Introduction

Palladium is often used as a catalyst in the synthesis of bulk pharmaceutical chemicals (BPC). Because palladium can remain in the BPC, its level needs to be rigorously monitored and controlled. Although Rousselet and Thuillier[1] suggested in 1979 that Pd levels should not exceed 20 μg g^{-1} or 20 ppm, an analytical method that can determine even lower levels of Pd is needed in today's pharmaceutical industry to assure consistent quality of BPC.

Atomic absorption spectroscopy (AAS) is a simple, reliable and selective analytical tool for the determination of metal contaminants. Rousselet and Thuillier [1] described the use of flame AAS to determine Pd in semi-synthetic penicillin. However, the limit of detection using flame AAS is generally higher than that using graphite furnace atomic absorption spectroscopy (GFAAS). Palaniappan et al. [2] recently described an indirect flameless AAS method for the determination of palladium in carbenicillin. However, the method required tedious sample preparations and large

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samples were needed to achieve the desired limit of detection (LOD). Although ICP-MS offers a superior LOD and a limit of quantitation (LOQ) of 0.1 μg g^{-1} has been reported for the determination of Pd in fosinopril sodium [3], the high cost of the ICP-MS instrument makes it impractical for routine quality control. GFAAS offers a lower LOD than flame AAS [4], a lower cost than ICP-MS, simple operation, and flexibility in the use of solvents for sample preparation. The determination of Pd by GFAAS has been applied to the analysis of geological samples [5,6].

This paper describes a GFAAS method for the direct determination of Pd in two different BPC, MK0476 and MK0462, and their synthetic intermediates, dicyclohexylamine (DCHA) salt of MK0476 and the tryptophol intermediate of MK0462. The direct dissolution of samples in concentrated (70%) nitric acid significantly simplifies sample preparation. The validation of this method, including verification of the absence of matrix effects caused by 70% nitric acid as well as studies of linearity, precision, accuracy, limit of detection and limit of quantitation, is presented.

2. Experimental

2.1. Reagents and materials

Concentrated nitric acid (70%, trace metal grade) was purchased from J.T. Baker (Phillipsburg, NJ). Certified palladium standard solution (1000 ± 3 μg ml^{-1} in 5% nitric acid) was purchased from High-Purity Standards (Charleston, SC). Deionized water was prepared by passing distilled water through a Milli-Q Water System (Millipore Corporation, Bedford, MA). The MK0476 and MK0462 BPC and their synthetic intermediates, DCHA salt of MK0476 and tryptophol of MK0462, were all obtained from Process Research and Development Department, Merck Research Laboratories (Rahway, NJ). The structures of these compounds are not pertinent to this paper and will be disclosed elsewhere.

2.2. Sample preparation

2.2.1. Preparation of standards

A 10 μg ml^{-1} Pd standard solution was prepared by diluting the 1000 μg ml^{-1} certified Pd standard with 2% nitric acid. The 2% nitric acid was prepared by diluting the 70% nitric acid with deionized water. Working standards containing 0.02, 0.05, 0.10, 0.30 and 0.50 μg ml^{-1} were prepared by diluting the 10 μg ml^{-1} standard solution with 2% nitric acid.

2.2.2. Preparation of samples

The samples of all BPC and their synthetic intermediates could be dissolved readily in 70% nitric acid owing to the strong oxidizing characteristics of nitric acid. An approximately 100 mg sample was weighed into a 10 ml volumetric flask, and then dissolved and diluted to volume with 70% nitric acid. The sample solutions of MK0476, MK0462 and DCHA salt of MK0476 were then analyzed directly without further dilution. Due to its higher Pd concentration, the sample solution of tryptophol of MK0462 was further diluted five-fold with 70% nitric acid to bring the Pd concentration of the sample into the linear working range.

2.3. Instrumentation

A Hitachi Z-8270 furnace atomic absorption spectrometer (Hitachi Instruments, Danbury, CT) was employed throughout the experiments. The spectrometer was equipped with Zeeman background correction capability using a permanent magnetic field which was applied to the furnace, with the field perpendicular to the optical axis. A pyrolytically-coated graphite tube with an integrated pyrolytically-coated platform (Hitachi Instruments) was used and heated longitudinally. Standards and samples were introduced using the attached model SSC-300 syringe autosampler. The instrumental parameters are listed in Table 1. The analytical signal was measured using peak height absorbance as in many cases this gives a better limit of detection [7].

Table 1
Instrumental parameters for the GFAAS method

Parameter	Setting
Absorption line (nm)	247.6
Slit width (nm)	0.40
Pd hollow cathode lamp current (mA)	4.0
Photomultiplier voltage (V)	450
Purging gas	Argon
Sample volume (μl)	20
Sample injection replicates	3
Peak measurement	Peak height absorbance

Temperature program:

Stage	Start (°C)	End (°C)	Ramp (s)	Hold (s)	Gas flow (ml min^{-1})
Drying	80	140	40	5	200
Ashing	700	700	–	50	200
Atomizing	2700	2700	–	10	30
Cleaning	2800	2800	–	4	200
Cooling				5	200

3. Results and discussion

3.1. Solvent selection

A critical step faced by the analytical chemist in atomic spectroscopy is sample preparation [8]. In most applications, the sample is dissolved in a suitable solvent before it is introduced into the instrument. Generally, a dilute (e.g. 2%) aqueous solution of an acid such as nitric acid is the preferred solvent for dissolving the samples. The aqueous nature of the solution provides ease of sample handling and the small amount of acid prevents the element of interest from precipitating or being adsorbed on the glass wall of the volumetric flask. However, the BPC and intermediates involved in this work are all solids that are insoluble in 2% nitric acid.

One approach to this problem is to digest the samples in acid using the microwave digestion technique, followed by diluting the digested samples with 2% nitric acid. However, this requires extra time and sample handling to complete the digestion and sample preparation procedure.

Another approach is to directly dissolve the sample in a solvent other than 2% nitric acid. It was found that the compounds involved in this work were all readily soluble in 70% nitric acid. Therefore direct dissolution of the samples with 70% nitric acid was used to avoid the microwave digestion procedure. This in turn reduced the sample handling, sample preparation time and chance of cross-contamination.

GFAAS is more flexible compared with flame AAS, ICP emission or ICP-MS in the use of solvents for sample preparation, and samples prepared in 70% nitric acid can be easily tolerated by GFAAS. Since flame AAS, ICP emission or ICP-MS all involve the continuous nebulization of the sample solution, 70% nitric acid is too viscous to be run through the nebulizer and still maintain flow stability. Moreover, the concentrated acid is too abrasive to be run on the systems continuously. In GFAAS, however, no continuous sample nebulization is involved and only a small volume of sample solution is introduced. Therefore, the problem of flow instability associated with using concentrated nitric acid in flame AAS, ICP emission or ICP-MS does not affect GFAAS and the system erosion caused by 70% nitric acid is negligible in GFAAS. The long term experience of the present authors indicated that the graphite tube

Table 2
Comparison of standards prepared in 70% and 2% nitric acid

Concentration of Pd standard (μg ml^{-1})	Peak height absorbance of standard made in 70% nitric acid [a]	Peak height absorbance of standard made in 2% nitric acid [a]	Ratio [b]
0.020	0.0252	0.0255	0.99
0.050	0.0591	0.0580	1.02
0.100	0.1126	0.1216	0.93
0.300	0.3296	0.3329	0.99
0.500	0.5346	0.5232	1.02

[a] Mean of three replicate results.
[b] Ratio of peak height absorbance of standard in 70% nitric acid to peak height absorbance of standard in 2% nitric acid.

could be used for at least 300 heating cycles using the described method.

Unlike flame AAS, ICP emission or ICP-MS, in which the solvent used to prepare standards and samples must be the same in order to provide the same nebulization efficiency and keep the temperature of the flame or plasma the same for both standards and samples, GFAAS does not necessarily require the matching of solvents used for standards and samples because it does not involve sample nebulization and the solvents are totally evaporated during the drying and charring stage; therefore, the temperature in the atomizer is not affected by the solvents. In this paper, the standards were prepared in 2% nitric acid, instead of 70% nitric acid which was used to prepare samples, to avoid the unnecessary handling and disposal of surplus 70% nitric acid.

3.2. Method validation

3.2.1. Verification of the absence of matrix effects caused by 70% nitric acid

Since the samples were prepared in 70% nitric acid and the standards were prepared in 2% nitric acid, a study was carried out to verify the absence of a matrix effect caused by the 70% nitric acid. This was done by analyzing two sets of standards, one set prepared in 70% nitric acid and the other prepared in 2% nitric acid. The results obtained from both sets of standards are listed in Table 2. It is clear that the ratios of peak absorbance of standard in 70% nitric acid to peak absorbance of standard in 2% nitric acid are not significantly different from 1.00 at all concentration levels. This indicates that the standards made in 70% nitric acid give the same results as the standards made in 2% nitric acid and that there is no matrix effect. Fig. 1 compares the shapes of the peaks obtained from standards prepared in both 70% and 2% nitric acid at different Pd concentrations ranging from 0.02–0.5 μg ml^{-1}. The peak shapes at each concentration are identical, indicating the absence of a matrix effect.

3.2.2. Method linearity

The linearity of the method was evaluated by analyzing a series of Pd standards prepared at concentrations of 0.0, 0.02, 0.05, 0.10, 0.30 and 0.50 μg ml^{-1} in 2% nitric acid. Linear regression using the peak absorbance of the standards as the y axis and the concentration of the standards as the x axis gave the statistical results listed in Table 3. The results clearly demonstrate the linearity of the instrument response as a function of the Pd concentration over the entire concentration range studied, which corresponds to 0–50 ppm (μg g^{-1}) Pd in the solid samples of MK0476, MK0462 and DCHA salt of MK0476. In the case of analysis of tryptophol of MK0462, which involves sample dilution, the linear range of the method is extended to 250 ppm Pd in the solid sample.

3.2.3. LOD and LOQ

Ali [9] described the determination of the LOD in atomic absorption spectroscopy as the analyte concentration that yields an analyte signal equal

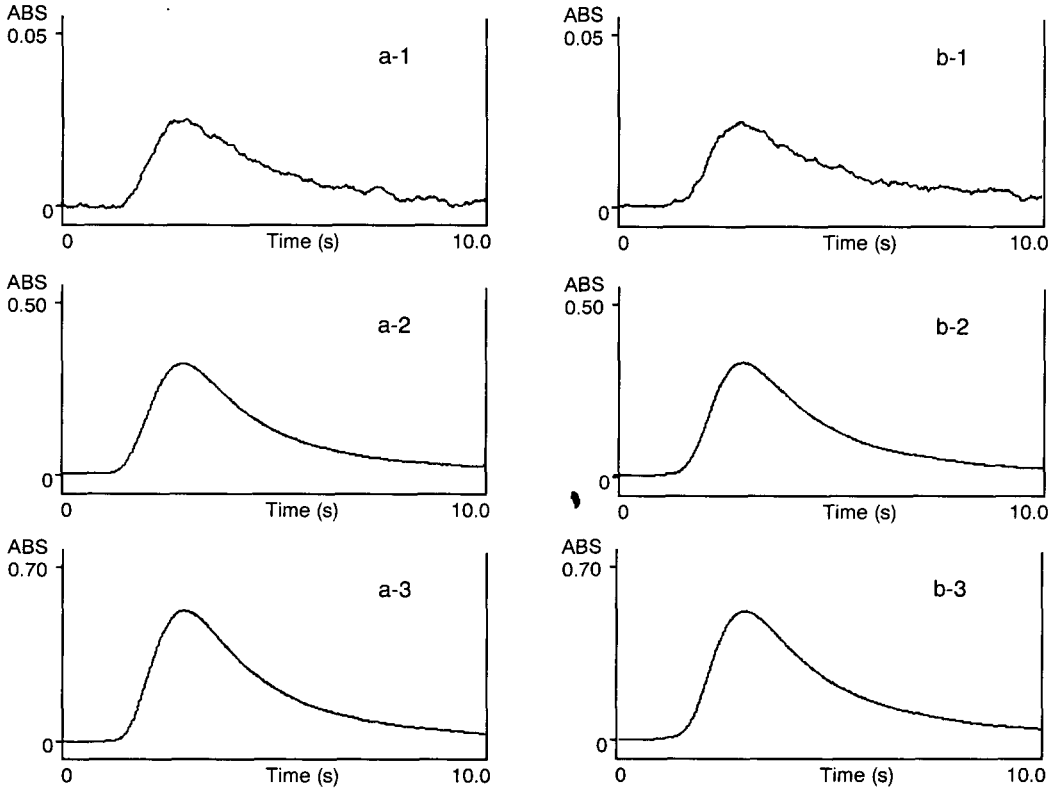

Fig. 1. Comparison of peak shapes obtained from standards made in 70% and 2% nitric acid. In 70% nitric acid: (a – 1) 0.02; (a – 2) 0.3; (a – 3) 0.5 μg/ml. In 2% nitric acid: (b – 1) 0.02; (b – 2) 0.3; (b – 3) 0.5 μg/ml.

to twice the standard deviation (σ) of a series of not less than 10 readings of instrument response taken close to the blank level. The standard deviation was used to estimate the noise level. In an article focusing on the LOD in atomic emission spectroscopy, Boumans [10] also gave the conventional definition of the LOD and the equation to calculate the LOD. He stated that the LOD (c_L) is experimentally defined as the analyte concentration that yields a net analyte signal equal to k times the standard deviation (σ_B) of the background:

$$c_L = k\sigma_B c_0 / x_A \quad (1)$$

where c_0 is the concentration yielding a net analyte signal x_A. For the sake of uniformity, Boumans recommended that the k value be three. Although Boumans' article was focused on atomic emission spectroscopy, the definition of the LOD was similar to that of Ali. When σ_B is substituted by σ, determined as described by Ali, Eq. (1) gives the LOD in atomic absorption spectroscopy.

In this paper, the LOD was determined as the

Table 3
Results of linearity study [a]

Slope	1.073
Intercept	0.008826
Standard error of slope	0.005719
Standard error of intercept	0.001387
Determination coefficient (r^2)	0.9999
Number of standards	6
Concentration range (μg ml^{-1})	0.0–0.5

[a] This statistical analysis was performed on a different data set than the one described in Table 2.

Table 4
Results of precision study

Number of replicate analysis	Peak absorbance			
	MK0476	MK0462	DCHA salt of MK0476	Tryptophol of MK0462
1	0.0270	0.0394	0.0238	0.2009
2	0.0284	0.0403	0.0259	0.2001
3	0.0269	0.0389	0.0255	0.2020
4	0.0250	0.0387	0.0265	0.2010
5	0.0254	0.0405	0.0248	0.2035
6	0.0271	0.0396	0.0267	0.2020
	Mean = 0.0266 SD = 0.00124 RSD (%) = 4.7	Mean = 0.0396 SD = 0.00073 RSD (%) = 1.8	Mean = 0.0255 SD = 0.00109 RSD (%) = 4.3	Mean = 0.2016 SD = 0.00119 RSD (%) = 0.6

concentration that yielded an analyte signal equal to three ($k = 3$) times the standard deviation (σ) of a series of 11 measurements of the peak absorbance of the lowest standard whose concentration c_0 was 0.02 μg ml^{-1}. After obtaining the standard deviation (σ) and mean peak absorbance (x_A) through experiment and substituting them along with c_0 and k into Eq. (1), the LOD was determined to be 0.007 μg ml^{-1}, which corresponded to 0.7 ppm (μg g^{-1}) in the solid samples of MK0476, MK0462 and DCHA salt of MK0476 using the described method. The LOQ was determined as the concentration that yielded an analyte signal equal to 10 ($k = 10$) times the standard deviation of the 11 measurements of the peak absorbance of the lowest standard. Substituting the k value in Eq. (1) with 10 gave the LOQ as 0.02 μg ml^{-1}, which corresponded to 2 ppm in the solid samples of MK0476, MK0462 and DCHA salt of MK0476. Due to the five-fold dilution of the sample of tryptophol of MK0462, the LOD and LOQ for this synthetic intermediate were determined to be 3.5 ppm and 10 ppm respectively in the solid sample.

3.2.4. Precision

The precision of the method was determined by repeated analysis of various samples and by evaluating the degrees of reproducibility among the replicate results from the same sample. To take the sample matrix into account, sample solutions containing MK0476, MK0462, DCHA salt of MK0476 and tryptophol of MK0462 were used instead of using a standard. Since the samples of MK0476, MK0462 and DCHA salt of MK0476 used in this study all had Pd levels lower than the LOQ (0.02 μg ml^{-1}), they were all spiked with Pd to bring the Pd concentration in the sample solutions to 0.02–0.03 μg ml^{-1}, so that the precision study could be done at a level equal to or slightly above the LOQ. The precision for the determination of tryptophol of MK0462 was studied at a typical Pd level using a non-spiked sample as it contained a higher level of Pd (0.2 μg ml^{-1}).

Repeated analysis of each sample gave the peak absorbance values listed in Table 4. The results—$< 5\%$ RSD for MK0476, MK0462 and DCHA salt of MK0476 samples—indicated good precision of the method for these samples at the Pd level close to the LOQ. A better % RSD value for tryptophol of MK0462 was achieved due to the higher level of Pd present in the sample.

3.2.5. Accuracy

The accuracy of the method was demonstrated by the recovery of a known amount of Pd spiked into various sample matrices including MK0476, MK0462, DCHA salt of MK0476 and tryptophol of MK0462. To demonstrate the accuracy of the

Table 5
Results of the recovery study

Parameter	Sample			
	MK0476	MK0462	DCHA salt of MK0476	Tryptophol of MK0462
Level of Pd spiked (μg ml^{-1})	0.02	0.02	0.02	0.2
Recovery [a] (%)	100	94	109	104

[a] Mean of duplicate determinations.

method at levels close to the LOQ, the sample solutions of MK0476, MK0462 and DCHA salt of MK0476 were spiked with Pd at 0.02 μg ml^{-1}, which represented the LOQ. The sample of tryptophol of MK0462 which contained 0.2 μg ml^{-1} Pd was spiked with 0.2 μg ml^{-1} Pd to demonstrate the accuracy of the method at higher Pd level. Table 5 summarizes the recovery results for the various samples and demonstrates excellent accuracy of the method for these samples.

3.3. Application of the method to real samples

Multiple batches of MK0476, MK0462, DCHA salt of MK0476, and tryptophol of MK0462 have been analyzed using the described method. The Pd levels in DCHA salt of MK0476 ranged from non-detected to 4 ppm and the Pd levels in MK0476 BPC ranged from non-detected to 2 ppm. Although the tryptophol of MK0462 had Pd levels ranging from 100–130 ppm, the Pd present in MK0462 BPC was not more than 4 ppm.

4. Conclusion

The determination of palladium using the described GFAAS method is simple, sensitive, accurate and relatively inexpensive. The direct dissolution of samples with 70% nitric acid provides simple sample preparation without adverse effects. The method covers the range up to 50 ppm Pd in the solid samples of MK0476, MK0462 and DCHA salt of MK0476, and up to 250 ppm Pd in the sample of tryptophol of MK0462. The LOD and LOQ were determined to be 0.7 ppm and 2 ppm respectively in the solid samples of MK0476, MK0462 and DCHA salt of MK0476.

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