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Determination of ruthenium in pharmaceutical compounds by graphite furnace atomic absorption spectroscopy

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Abstract

A graphite furnace atomic absorption (GFAA) spectrometric method for the determination of ruthenium (Rh) in solid and liquid pharmaceutical compounds has been developed. Samples are dissolved or diluted in dimethyl sulfoxide (DMSO) without any other treatment before they were analyzed by GFAA with a carefully designed heating program to avoid pre-atomization signal loss and to achieve suitable sensitivity. Various inorganic and organic solvents were tested and compared and DMSO was found to be the most suitable. In addition, ruthenium was found to be stable in DMSO for at least 5 days. Spike recoveries ranged from 81 to 100% and the limit of quantitation (LOQ) was determined to be 0.5 $\mu g\, g^{-1}$ for solid samples or 0.005 $\mu g\, ml^{-1}$ for liquid samples based a 100-fold dilution. The same set of samples was also analyzed by ICP-MS with a different sample preparation method, and excellent agreement was achieved.

Keywords: GFAA; Ruthenium; Pharmaceutical compounds

1. Introduction

Ruthenium compounds are commonly used as catalysts in the pharmaceutical industry. It is a known fact that high levels of ruthenium damages human lungs and eyes. Therefore, monitoring and controlling the levels of ruthenium in drug substances are critical. A number of methods have been reported for the determination of ruthenium(III) (Ru III), using spectrophotometry [1], voltammetry [2,3], atomic absorption spectrometry [4–14], and flow injection catalytic method [15].

Atomic absorption and atomic emission spectrometry can be used for the analysis of total elemental content in the samples regardless of the chemical forms of these elements. Early reports on the analysis of ruthenium catalysts [5], alloys [6], and ruthenium complexes [7] by atomic absorption techniques pointed out poor sensitivity and severe matrix interferences as the most prominent concerns in its determination. This is true in both flame and graphite furnace atomic absorption (GFAA). Although graphite furnace atomic spectroscopy offers better sensitivity, severe physical, chemical, and spectral inferences from sample matrix [6] and coexisting metals [5,6,8] also make accurate and precise quantitative analysis of Ru a challenging task.

Various matrix modifiers [5,6,9] were studied in an effort to improve the determination of ruthenium by GFAA. It was reported by Scaccia and Goszczynska [5] that the presence of platinum in the sample solution increased ruthenium sensitivity. It was found by Scarborough [6] that the addition of high level of uranium could result in the elimination of the interferences from metals such as Mo, Rh, and Pd. El-Defrawy et al. [9] reported the use of potassium cyanide to eliminate sample matrix effects and to greatly enhance the absorption signal.

In the pharmaceutical industry, the determination of residual amounts of ruthenium, if it has been used in any of the synthesis processes, in drug substances or their intermediates is mandatory due to its potential toxicity. Frequently, pharmaceutical compounds are not soluble in dilute acid and a microwave digestion system is frequently needed for sample preparation. Methods have been developed in the authors' laboratory using either concentrated nitric acid [16] or organic solvents [17] to dissolve the samples directly for "dissolve-and-shoot" GFAAS determination. Both methods are simple, accurate and free of cross-contamination. Ruthenium forms volatile

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ruthenium tetra-oxide at 108 °C which can potentially be lost at drying and/or charring stages if experimental conditions are not optimized properly. In addition, the high melting point (2334 °C) and boiling point (4150 °C) of ruthenium [18] also makes this element very difficult to atomize.

A simple interference-free GFAAS method for Ru analysis in pharmaceutical compounds has been developed. The pharmaceutical entities involved are either diisopropanolamine (DIPA) or trimethylglycine (TMG) salts and their acyl acetate solutions. Samples are dissolved directly in dimethyl sulfoxide (DMSO) without any other treatment and graphite furnace atomic absorption (GFAAS) determination of ruthenium was performed using carefully designed heating programs. Results are compared favorably with those obtained with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis.

2. Experimental

2.1. Reagents and materials

Trace metal grade hydrochloric acid and concentrated nitric acid, ACS certified DMSO, HPLC grade methanol, and acetonitrile were purchased from Fisher Scientific (Fair Lawn, NJ). Ru stock standard solution (1000 μg ml^{-1}) was bought from High-Purity Standards (Charleston, SC). Deionized water was prepared by passing distilled water through a Hydro Ultrapure Water System (Garfield, NJ). The compounds used in the experiments were from Merck Research Laboratories (Merck & Co. Inc., Rahway, NJ). The structures of the compounds are not relevant to the analysis, and thus, are not released.

2.2. Preparation of standards and samples

2.2.1. Standard and sample preparation for GFAA analysis

The calibration blank used is DMSO. The 10 μg ml^{-1} Ru intermediate standard was prepared by diluting the stock 1000 μg ml^{-1} Ru standard with 5% HCl solution. Ruthenium standards in various acid solutions or organic solvents were made by diluting the 10 μg ml^{-1} Ru intermediate standard with the corresponding acid solution or organic solvents.

Solid samples were prepared by accurately weighing 5–100 mg of samples into 10 ml volumetric flasks before they were dissolved and diluted to volume with DMSO. Liquid samples were prepared by diluting aliquots of the sample solutions with DMSO directly.

2.2.2. Standard and sample preparation for ICP-MS analysis

Analysis of Ru by ICP-MS was performed in 80% nitric acid solution. The calibration blank, the 80% nitric acid solution, was prepared by diluting the concentrated nitric acid with deionized water. The Ru working standards of 10, 20, and 50 ng ml^{-1} in 80% nitric acid solution were prepared by the dilution of the 10 μg ml^{-1} Ru intermediate standard solution.

Solid samples were prepared by accurately weighing approximately 10 mg of samples into 10 ml volumetric flasks before they were dissolved and diluted to volume with 80% nitric acid solution. Liquid samples were prepared by diluting aliquots (≤100 μl) of the sample solution with 80% nitric acid solution directly.

Table 1
The GFAA instrument operating condition and measurement parameters

Wavelength (nm)	349.9
Slit width (nm)	0.2
Signal type	Peak area
Background correction	Zeeman
Sample volume (μL)	30
Measurement time (s)	3
Number of replicates	2
Purging gas	Argon

Furnace temperature program

Phase	Temperature (°C)	Time (°C)	Ramp (°C/s)	Gas flow (l/min)	Commands
1	100	5	3	0.3	
2	180	5	2	0.3	
3	1500	10	150	0.3	
4	2750	3	0	Off	RD TC
5	2850	3	0	0.3	TC
6	100	5	0	0.3	
7	2850	3	0	0.3	TC
8	100	5	0	0.3	
9	2850	3	0	0.3	TC

2.3. Instrumentation

TJA Solaar GF95 Atomic Absorption Spectrometer with Zeeman background correction was used in this study. Extended Life Cycle (ELC) type platform graphite tube (TJA, Cambridge, UK) was used. The instrument operating conditions are listed in Table 1.

Perkin-Elmer Elan 6000 ICP-MS was used for the determination of Ru in the selected pharmaceutical compounds to compare the results and validate the GFAA method. The Perkin-Elmer Elan 6000 is equipped with a 40 MHz radio frequency (RF) generator, Meinhard nebulizer with cyclonic spray chamber, and platinum sampler and skimmer cones. $^{99}Ru^+$, $^{101}Ru^+$, and $^{102}Ru^+$ are all monitored to ensure interference-free quantitation.

3. Results and discussions

3.1. Selection of a suitable solvent for Ru atomic absorption determination

In order to evaluate the effects of various solvents on ruthenium atomic absorption signals, 50 ng ml^{-1} Ru standards in 5% HCl, concentrated HCl, 5% HNO_3, concentrated HNO_3, DMSO, acetonitrile (MeCN), and methanol (MeOH) were made and analyzed by GFAAS. The results are given in Table 2. It is obvious that the atomic absorption (AA) signals of Ru in both 5% nitric acid and concentrated nitric acid solutions are much smaller than those in other solutions, and the absorption of Ru in concentrated

Table 2
The comparison of atomic absorption signals (A) and standard deviation (S.D.) of 50 ng ml^{-1} Ru in different solvents

	5% HCl	Conc. HCl	5% HNO_3	Conc. HNO_3	DMSO	MeCN	MeOH
A	0.1012	0.0992	0.0470	0.0181	0.0996	0.0928	0.1130
S.D.	0.0002	0.0018	0.0009	0.0060	0.0016	0.0022	0.0026

nitric acid is smaller than that in 5% nitric acid solution, as shown in Table 2. The low absorption signals can be easily explained by the formation of volatile ruthenium tetra-oxide (RuO_4) which is lost during drying and charring stages in the presence of oxidizing nitric acid before reaching atomization stage.

The atomic absorption signals of 50 ng ml^{-1} Ru standard in DMSO, MeOH, and MeCN are comparable (Table 2), with the sensitivity in MeOH slightly higher and that in MeCN slightly lower.

Neither 5% nor concentrated HCl is suitable solvent for Ru determination since none of the compounds tested are soluble in them. On the other hand, although excellent in dissolving all compounds tested, concentrated nitric acid cannot be used for ruthenium determination either, since the oxidizing nature of the nitric acid will lead to the formation of ruthenium tetra-oxide and thus the loss of analyte before atomization.

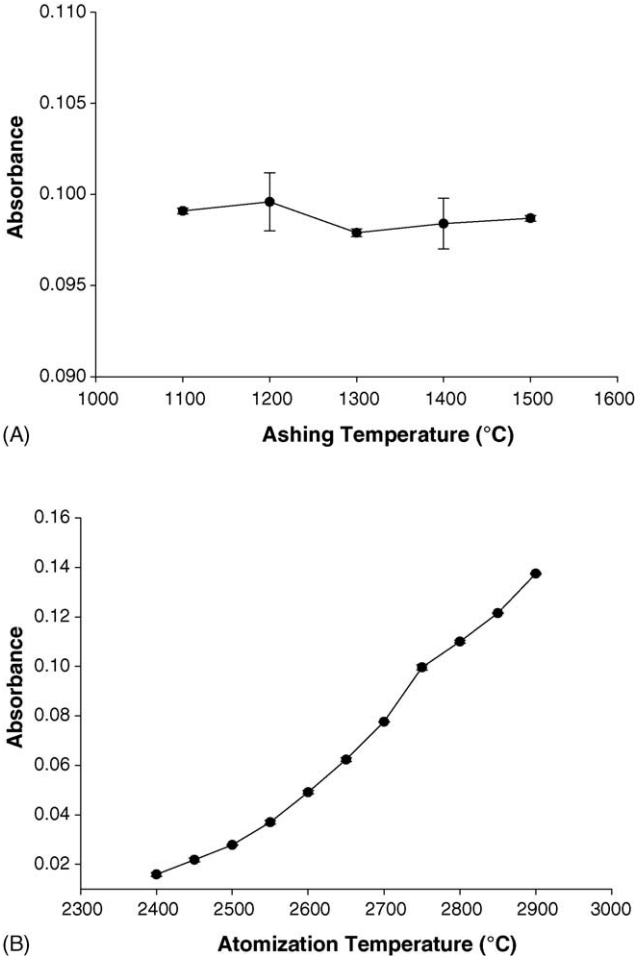

Fig. 1. (A) Optimization of ashing temperature and (B) optimisation of atomization temperature.

All compounds tested also have excellent solubility in MeOH, MeCN, and DMSO. Whereas, it was observed that precipitation took place immediately after trace amount of acid was introduced into the samples dissolved in MeOH, which makes matrix spiking impossible since the stock ruthenium standard was made in 10% HCl. MeCN cannot be used as a suitable solvent for this analysis due to its high volatility, thus leaving DMSO the only choice for this purpose.

3.2. Optimization of ashing and atomization temperatures

Ashing temperature of Ru in DMSO was optimized over a range of 1100–1500 °C using a 50 ng ml^{-1} ruthenium standard at a constant atomization temperature of 2750 °C. As shown in Fig. 1, no significant change in ruthenium absorption was observed over the temperature range, and thus the ashing temperature was set at 1500 °C thereafter to ensure maximum matrix removal without signal loss.

The atomization temperature of Ru in DMSO was studied over the range of 2400–2900 °C using the same 50 ng ml^{-1} ruthenium standard at a constant ashing temperature of 1500 °C. Within this range, the atomic absorption signal keeps improving with increasing atomization temperature without reaching a plateau up to 2900 °C (Fig. 1B). At 2750 °C, the atomic absorption signal of Ru is about 0.1, which is sufficient to meet the sensitivity requirement of Ru in the samples. Although the sensitivity could be enhanced with higher atomization temperatures, the trade-off is the compromised lifespan of graphite furnace tubes. In addition, higher temperature than 2750 °C might not be achievable on some atomic absorption spectrometers made by different manufacturers located on other sites of the company,

Table 3
The analysis of Ru in pharmaceutical compounds by GFAA and ICP-MS

Compound	GFAAS Ru ± S.D. (μg g^{-1})	Recovery (GFAAS) (%)	ICP-MS Ru ± S.D. (μg g^{-1})
A	4.2 ± 0.1	87	3.9 ± 0.1
B	64.9 ± 1.9	90	60 ± 1.0
C	6.3 ± 0.2	89	6.0 ± 0.5
D	33.4 ± 0.7	91	32.7 ± 1.2
E	8.9 ± 0.1	81	9.0 ± 0.6
F	36.8 ± 0.3	82	37.7 ± 1.5
G	60.0 ± 1.9	97	57.1 ± 1.4
H	208 ± 4	100	195 ± 12
I[a]	10.4 ± 0.2	100	10.1 ± 1.0
J	2.2 ± 0.1	90	2.3 ± 0.1
K[a]	<0.1	98	<0.1
L	229 ± 6	94	228 ± 9
M	181 ± 4	92	174 ± 6
N	13.2 ±0.1	99	12.8 ±0.3

[a] Liquid samples.

Table 4
Stability of Ru standards (ng ml^{-1}) in DMSO

Time (h)	Prepared Rh value (ng ml^{-1}) in DMSO					
	50		100		200	
	Measured value	Recovery (%)	Measured value	Recovery (%)	Measured value	Recovery (%)
24	47	94	99	99	189	95
72	49	98	96	96	185	93
120	48	96	101	101	189	95
144	49	98	92	92	173	87

and thus making method transfer to other sites impossible. An atomization temperature of 2750 °C was used throughout this study.

Due to its high boiling point, a memory effect is commonly seen in Ru analysis by GFAA. The graphite furnace tube was cleaned at 2850 °C with the argon protection gas turned on after the atomization step followed by a cooling step to 100 °C (Table 1). This "cleaning" step was repeated three times and proved to be very effective in mitigating the memory effect.

3.3. Validity and accuracy of the method

DMSO, well known as a non-volatile solvent for many organic compounds, is widely used in the pharmaceutical industry for sample dissolution. After the solubility and stability of the 14 samples were confirmed, they were dissolved directly in DMSO without any other treatment before GFAA analysis. In order to assess the validity of this method, 50 ng ml^{-1} of Ru was spiked to each of the 14 samples dissolved in DMSO to evaluate the spike recoveries. The results of the 14 samples from the GFAAS analysis were also compared to those from the ICP-MS analysis. The results are summarized in Table 3. The spike recoveries range from 81 to 100%, and the Ru results obtained by GFAAS in both the liquid and the solid samples compare favorably with those obtained by ICP-MS.

3.4. Stability of ruthenium in DMSO

Commercial Ru stock standard is normally stored in 10% HCl for long term stability. Although proven to be an excellent solvent for this study, the stability of Ru in both standards and samples prepared in DMSO is unknown. Since both sample and standard storage are often unavoidable, a stability study is warranted to ensure their integrity.

To this end, Ru standards of 50, 100, and 200 ng ml^{-1} were prepared by diluting the 10 μg ml^{-1} intermediate Ru standard made in 5% HCl with DMSO. These Ru standards were analyzed immediately (0 h), after 24, 72, 120, and 144 h, respectively. The measured GFAA signal intensities obtained with the aged standards are compared to those obtained with the freshly made (0 h) standards and the results are compiled in Table 4. It is clearly evident that Ru in DMSO is stable for at least 5 days without significant loss.

Table 5
LOD and LOQ of the method

Replicates	Measured Ru concentration (ng ml^{-1})
1	9.188
2	9.325
3	9.377
4	8.368
5	8.962
6	9.168
7	8.873
8	8.776
9	8.455
10	8.051
11	9.791
Mean	8.94
S.D.	0.51
LOD	1.52
LOQ	5.06

3.5. Limit of detection and linearity

Limit of detection (LOD) and limit of quantitation (LOQ) for ruthenium in DMSO were estimated by analyzing 11 replicate aliquots of the spiked calibration blanks as 11 samples at concentrations between two and five times the estimated limit of detection (based on the standard deviation of 11 replicate blanks). Mathematically, the LOD and LOQ are defined as 3 and 10 times of the standard deviation of the 11 measurements, respectively. The resulting LOD and LOQ for ruthenium in DMSO are 1.5 and 5.1 ng ml^{-1}, respectively, as shown in Table 5. The LOD and LOQ for solid sample are then 150 and 510 ng g^{-1} based on a 100 mg sample dissolved in 10.0 ml DMSO, which are much lower than the LOD and LOQ set by the project.

To determine the linear range, a series of standards above the upper calibration range, from 300 to 450 ng ml^{-1} were analyzed as samples. The upper linear range, defined as being the highest concentration for which the result (in concentration units) is within ±10% of the true (prepared) value, was determined to be 350 ng ml^{-1}. Using this method, ruthenium in the solid samples can then be determined without dilutions from 0.5 to 35 μg g^{-1}.

4. Conclusions

Using a proper heating program with the atomization temperature set at 2750 °C and ashing temperature at 1500 °C, ruthenium in drug substances dissolved in or diluted with DMSO

can be determined accurately without interferences with matrix-matched standards by graphite furnace atomic absorption spectrometry. Liquid samples can be determined without dilution up to 350 ng ml^{-1}, and solid samples can be determined from 0.5 to 35 μg g^{-1}.

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