Application Note

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Method Verification of the United States Pharmacopeia Monograph with the Agilent Cary 60 UV-Vis

Quantification of iron impurities in magnesium sulfate following the USP monograph

Authors

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Abstract

This study outlines how the Agilent Cary 60 UV-Vis spectrophotometer can be used to verify the United States Pharmacopeia (USP) monograph for the quantification of iron impurities in magnesium sulfate. The Cary 60 was fitted with an 18-cell multicell changer, enabling automated analysis of blanks, standards, and samples under the control of the Agilent Cary WinUV software. The verified method was used to determine the relative weight of iron in two magnesium sulfate samples below the specified limit of 0.5 µg/g. The results confirm the suitability of the Cary 60 for pharmaceutical-related quality control applications.

Introduction

Government agencies at national and international levels regulate pharmaceuticals to ensure the safety, efficacy, quality, and labeling of drugs. These agencies publish pharmaceutical monographs for drug developers and manufacturers, helping them to maintain consistent quality standards throughout the research, development, and manufacturing processes. Major pharmacopoeias, such as the USP, European Pharmacopoeia (Ph. Eur.), British Pharmacopoeia (BP), Japanese Pharmacopoeia (JP), and Pharmacopoeia of the People's Republic of China (ChP) publish pharmaceutical monographs as part of their official compendia. Monographs provide methods for the quantitative determination of pharmaceutical substances, including the measurement of impurities within acceptable limits, and are essential for compliance with regulatory requirements. UV-Vis spectroscopy is a commonly specified technique in various pharmaceutical monographs.

According to good manufacturing practices (GMP), any techniques that are used to analyze pharmaceutical substances must be evaluated to determine their suitability under actual conditions of use. The USP provides general information on the verification of compendial procedures in USP General Chapter <1226> Verification of Compendial Procedures and specific guidance on UV-Vis spectroscopic techniques in USP General Chapter <857> Ultraviolet-Visible Spectroscopy.[1,2]

In a previous application note, the Cary 60 UV-Vis spectrophotometer and Cary WinUV software performed the measurements that are required by many of the verification tests outlined in USP <857>.[3] This study provides an example of how the Cary 60 UV-Vis fitted with an 18-cell multicell changer can be used for method verification for the quantification of iron in magnesium sulfate per the USP Magnesium Sulfate monograph.[4]

The Cary 60 UV-Vis

The Cary 60 UV-Vis is a flexible, powerful, and reliable UV-Vis system that is ideal for the routine analysis of a range of substances in pharmaceutical laboratories (Figure 1). The Cary 60 UV-Vis combines high-quality data collection with low cost of ownership, primarily because of its powerful xenon lamp light source. The robustness and reliability of the lamp, as reflected by its 10-year guarantee, reduces any potential replacement and revalidation costs, and maximizes instrument uptime.

The Cary 60 UV-Vis has been independently audited for its environmental impact and has received the ACT (Accountability, Consistency, Transparency) label, verified by My Green Lab. The label provides information about the environmental impact of the Cary 60 UV-Vis throughout its entire life cycle.

The Cary 60 UV-Vis improves the environmental impact of laboratories without impeding productivity or scientific progress.

Figure 1. The Agilent Cary 60 UV-Vis spectrophotometer includes a 10-year replacement warranty for the xenon lamp.

Experimental

Reagents

- **Dilute hydrochloric acid (HCl)**: 1 mL of HCl in 1,000 mL of water
- **Solution A**: 500 mg/mL of ammonium acetate in water
- **Solution B**: 13.4 mg/mL of ascorbic acid in water
- **Color reagent**: 3.8 mg/mL of 3-(2-pyridyl)-5,6-di-(2-furyl)-1,2,4-triazine-5',5"-disulfonic acid, disodium salt
- **Standard iron stock solution**: 1.0 µg/mL iron in dilute HCl

Preparation of blank, standard, and sample solutions

The USP Magnesium Sulfate monograph[4] describes the quantification of the relative mass of iron in a magnesium sulfate sample calculated from a line of best fit, based on the analysis of three known standard solutions by UV-Vis.

All glassware was washed with dilute HCl. Three standards were prepared by transferring 2.0, 5.0, and 10.0 mL of the standard iron stock solution to separate 50 mL volumetric flasks and diluting them to 35 mL with dilute HCl. A blank solution was prepared by transferring 35 mL of dilute HCl into a 50 mL volumetric flask. All stock solutions were prepared on the day of the tests.

Two sample solutions were prepared by transferring 10 g of magnesium sulfate (CAS number 7487-88-9) into a 50 mL volumetric flask and diluting to 35 mL with dilute HCl. The concentration of iron in the sample solutions was determined using the established matrix-matched calibration curve.

Table 1. Standards used for calculating the relative mass of iron in magnesium sulfate.

Standard	Concentration of Iron (mg/L)	Mass of Iron in Standard (µg)
Standard 1	0.04	2
Standard 2	0.1	5
Standard 3	0.2	10

Instrumentation

The Cary 60 UV-Vis was fitted with an 18-cell multicell changer and controlled using Cary WinUV software. The multicell changer can be moved automatically so that each cuvette can be positioned in turn, enabling the automated measurement of up to 18 cuvettes. The multicell therefore enabled multiple standards, samples, and repeats to be run unattended, using one method, greatly improving productivity.[5] Measurements were taken with 1 cm pathlength cells at room temperature.

The Cary WinUV Concentration application software was used to collect blank, standard, and sample measurements by creating a Concentration method. The method automatically performed blank subtraction, established a concentration curve, and calculated the concentration of iron in the samples. Method parameters are described in Table 2.

Table 2. Agilent Cary WinUV Concentration application parameters used for the Agilent Cary 60 UV-Vis method.

Parameter	Setting
Analysis Wavelength	594 nm*
Signal Averaging Time (SAT)	0.5 s
Spectral Bandwidth (SBW)	1.5
Number of Standards	3
Number of Samples	2

* Specified in the USP Magnesium Sulfate monograph[4]

Results and discussion

Method verification

The process of verification of UV-Vis methods, which is described in USP <857>[2], includes the following requirements:

Measurement accuracy: Measurement accuracy can be determined through recovery studies of known concentrations of the analyte within the required matrix.

The measurement accuracy of the iron in magnesium sulfate test was determined by spiking three standard samples of magnesium sulfate with a nominal concentration of 0.1 mg/L with 2, 4, and 6 mL of 0.2 µg/mL standard solutions of iron. The concentration of iron was then calculated by collecting six repeat measurements (replicates) and calculating the mean absorbance for each spiked solution. The percent recovery was then calculated using Equation 1.

Equation 1.

$$\%\text{Recovery} = \frac{C_1}{C_2} \times 100$$

Where:

C_1 = measured concentration

C_2 = calculated concentration

The recovery results of 100 ± 6% of the expected concentration of iron in magnesium sulfate (Table 3) were well within the acceptable limits of 80.0 to 120.0% prescribed by USP <857> for mean recovery in impurity analysis.

Table 3. Recovery analysis of three magnesium sulfate samples spiked with an iron standard (0.2 mg/L) at three concentrations using an Agilent Cary 60 UV-Vis spectrophotometer.

Magnesium Sulfate Accuracy Sample	Nominal Conc. (mg/L)	Volume of Spike Standard (mL)	Calculated Conc. (mg/L)	Measured Conc. (mg/L)	Recovery (%)
1	0.100	2	0.1166	0.1104	94.6
2		4	0.1286	0.1218	94.8
3		6	0.1375	0.1403	102.0

Measurement precision: Precision or repeatability can be determined by measuring the concentration of six similarly prepared sample solutions at 100% of the assay test concentration and determining their relative standard deviation (RSD).

The precision of the iron in magnesium sulfate test was determined by splitting a standard solution with a nominal concentration of 10 µg of iron in 50 mL of the analysis matrix into six portions. The absorbance of the six portions loaded in the 18-cell changer was then measured at 594 nm using the Cary 60 UV-Vis. Using the Sample Averaging function in the WinUV Concentration application software, all six replicate samples were measured within the same run. The repeatability of the measurements was assessed by calculating the RSD of the measured concentration of each sample. The RSD was determined to be 1.8%, which is within the limit of not more than (NMT) 15.0 to 20.0%, prescribed by USP <857>.

Table 4. Precision measurements of iron in six replicate samples of magnesium sulfate.

Sample	Absorbance	Sample Concentration (mg/L)
Sample 1-1	0.1864	0.1894
Sample 1-2	0.1884	0.1924
Sample 1-3	0.1929	0.1993
Sample 1-4	0.1898	0.1946
Sample 1-5	0.1881	0.1920
Sample 1-6	0.1875	0.1911
Standard Deviation (SD)	0.002276	0.00348
%RSD	1.205	1.802113

Specificity: Specificity can be ensured by demonstrating a lack of interference in the spectral range from the standard and sample matrix.

Wavenumber scans of a blank and a standard iron solution with a nominal concentration of 0.2 mg/L were performed in the Cary WinUV Scan application software. Figure 2 shows a comparison of the photometric response of the color reagent (3-(2-pyridyl)-5,6-di-(2-furyl)-1,2,4-triazine-5',5"-disulfonic acid, disodium salt) in an iron solution compared to the same solution with no iron (blank solution) around the targeted peak at 594 nm. The blank solution shows no photometric response in the analysis spectral range, indicating no interferences in this range.

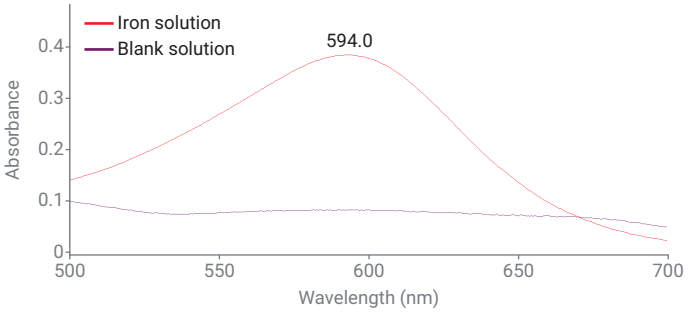

Figure 2. Comparison of wavelength scans between a color reagent added to an iron-containing solution and the same reagent added to a blank solution (no iron).

Quantitation limit (QL): The QL of iron was estimated by calculating the SD of six replicate measurements of a blank solution and multiplying the value by 10. For this test, the estimated QL was calculated to be 0.00231 mg/L. To confirm this estimated quantitation limit, the signal-to-noise ratio of this measurement at the limit should be greater than 10 and the measurement at this concentration must be determined to be accurate and precise. Ultimately, the QL should be ≤ 50% of the specified limit in the monograph to be considered valid.

Table 5. Calculated concentrations of six replicate measurements of a blank solution and the estimated QL of iron.

Replicate	Concentration (mg/L)
1	0.00776
2	0.0075
3	0.00776
4	0.00802
5	0.00762
6	0.00736
SD	0.000231
QL	0.002319

Given the successful verification of the Cary 60 UV-Vis procedure, the analysis of iron in magnesium sulfate per the USP monograph can be conducted with confidence. The verification confirms that the instrument and experimental parameters are suitable for actual conditions of use.

Measurement of iron in magnesium sulfate: To determine the relative mass of iron in magnesium sulfate, the USP monograph specifies that the absorbance values of three standard solutions of iron in the analysis matrix should be used to create a calibration curve. The relative mass of iron in a sample of magnesium sulfate can then be determined using Equation 2.

To prepare the analysis matrix, 5 mL each of Solution B and the color reagent were added to each of the standard, sample, and blank solutions (see Experimental section). Each solution was then made up to volume (50 mL) with dilute HCl.

The absorbance of each standard solution was measured against the blank solution and plotted against their iron content, as shown in Figure 3.

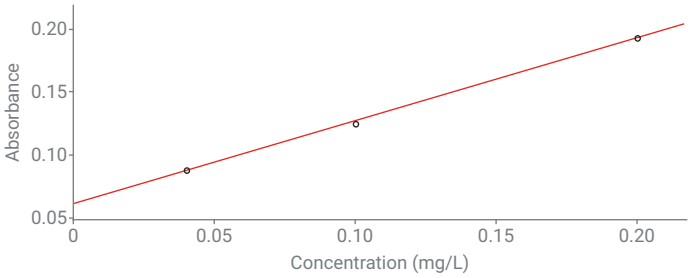

Figure 3. Calibration curve based on the measurement of three matrix-matched iron standard solutions with an Agilent Cary 60 UV-Vis.

The absorbance values of two sample solutions were then measured and the readings were used to calculate the iron content relative to the mass of magnesium sulfate, according to Equation 2.

Equation 2.

$$\text{Result} = \frac{C}{W}$$

Where:

C = content of iron in sample solution (µg)

W = weight of magnesium sulfate in sample solution

Table 6. Relative iron content in two magnesium sulfate samples.

Magnesium Sulfate Sample	Content of Iron (µg)	Result (µg/g)
Sample 1	3.1	0.31
Sample 2	2.5	0.25

The results for iron fall within the acceptance criteria of NMT 0.5 µg/g, which is the upper limit specified in the USP monograph.

Conclusion

An Agilent Cary 60 UV-Vis with an 18-cell changer was used to demonstrate how a UV-Vis compendial procedure from a USP monograph can be verified. The Agilent Cary WinUV software and 18-cell changer automated the multiple replicate measurements required by the USP validation tests, reducing the workload of the operator. The validation tests included confirmation of measurement accuracy, precision, specificity, and determination of the quantitation limit.

After verifying the method, the Cary 60 UV-Vis was used to measure the content of iron in two magnesium sulfate samples following the USP Magnesium Sulfate compendial procedure. The results were within the acceptance criteria for that material of < 0.5 µg/g.

The Cary 60 UV-Vis is ideal for routine pharma quality assurance/quality control (QA/QC) workflows and R&D applications. Optional Agilent Cary WinUV Pharma software is also available that supports compliance with US FDA 21 CFR Part 11, EU Annex 11, and similar national electronic record regulations.

References

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Further information

- Cary 60 UV-Vis Spectrophotometer
- Cary WinUV Software for UV-Vis Applications
- UV-Vis Spectroscopy & Spectrophotometer FAQ

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