Update to the UV-Visible ChemStation method for testing photometric accuracy in the ultraviolet region of the spectrum

Technical Note

Introduction

The photometric accuracy of a spectrophotometer is essential for ensuring correct results. The accuracy of a UV-Vis spectrophotometer is confirmed for the ultraviolet (UV) region of the spectrum using NIST-traceable 935a potassium dichromate in solution, and for the visible region of the spectrum using NIST-traceable 930e neutral density filters. These tests allow not only the accuracy of a spectrophotometer to be determined as required by the global pharmacopeia tests, but also to ensure that results can be compared between spectrophotometers, whether in the same laboratory or in different global locations.

The release of UV-Visible ChemStation (B.05.01) includes an update to the method used for determining photometric accuracy. This technical note describes the application of that test.

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Pharmacopeia requirements

Both the United States Pharmacopeia[1] (USP) and the European Pharmacopoeia[2] (EP) specify to test the photometric accuracy of a UV-Vis spectrophotometer using a solution of potassium dichromate[1,2]. While the EP specifies that potassium dichromate should be dissolved in 0.005 M sulfuric acid, the standards are available also dissolved in 0.001 M perchloric acid from NIST-traceable suppliers. The reason for using perchloric acid is the reduced risk of other complexes forming in the solution due to the greater ionic strength and greater salt effects of sulfuric acid[3].

For the USP, it is recommended that the absorbance of solutions of NIST-traceable 935a potassium dichromate be compared to the certified values at 235, 257, 313 and 350 nm. The acceptance criteria are ±1% A or ± 0.01 A, or whichever is larger. The EP describes dissolving 57.0 to 63.0 mg of NIST-traceable 935a potassium dichromate in 0.005 M sulfuric acid, or using suitable certified reference materials (CRMs). The EP provides specific absorbances and maximum tolerances which are shown in Table 1. The tolerances are based on an absorbance tolerance of ±0.01 A.

Table 1. Specific absorbance of potassium dichromate at the four referenced wavelengths as per the EP

Wavelength (nm)	Specific absorbance	Maximum tolerance
235	124.5	122.9–126.2
257	144.5	142.8–146.2
313	48.6	47.0–50.3
350	107.3	105.6–109.0

Rationale behind the change

Agilent's UV-Visible ChemStation software offers automatic measurements and data evaluation procedures within its Verification and Diagnostics mode. Previously, UV-Visible ChemStation (version B.04.02 and earlier versions) performed the 935a photometric accuracy test by comparing the normalized absorbance, based on the user-entered weight of potassium dichromate in the solution used, to the tolerances directly from the EP.

The weight of potassium dichromate could come from several sources; the actual weight measured in the laboratory or the reported weight on the CRM certificate. This presents a limitation where the weight of potassium dichromate solution used must be within the range allowed by the EP for the normalized tolerance range.

Most modern laboratories now source CRMs from companies that specialize in CRM production and that meet the relevant ISO /IEC 17025 and ISO Guide 34 procedure requirements. These CRMs are supplied with documentation on the certified values of the CRM and on the combined analytical and instrument uncertainties. For photometric accuracy 935a, the certified absorbance is reported for the four referenced wavelengths: 235, 257, 313 and 350 nm.

UV-Visible ChemStation (version B.05.01) has a change to the photometric accuracy 935a performance test. At the start of the test, the user can input data about the CRM that is being used for the test. Information entered includes the serial number, potassium dichromate weight (in mg), and the uncertainty for the performance test (in Absorbance Units, AU). Most importantly, the user can directly enter the certified absorbance of the CRM for each wavelength in AU. The lower and upper limits for the test are based on the entered certified absorbances and the entered uncertainty and are also reported in AU.

Measurement and evaluation

In the setup for verification, the photometric accuracy 935a test is chosen by selecting the checkbox for potassium dichromate. The user can enter the required information about the CRM being used (Figure 1).

A blank is performed on a solution of perchloric acid that is supplied with the CRM before the absorbance of the 935a potassium dichromate solution is acquired. The net measured absorbance at the four wavelengths, as set out by the pharmacopeia, is compared to the certified absorbance that was entered. A report is generated listing the upper and lower limits for the test, the measured and certified absorbances, and indicating a Pass or Fail result.

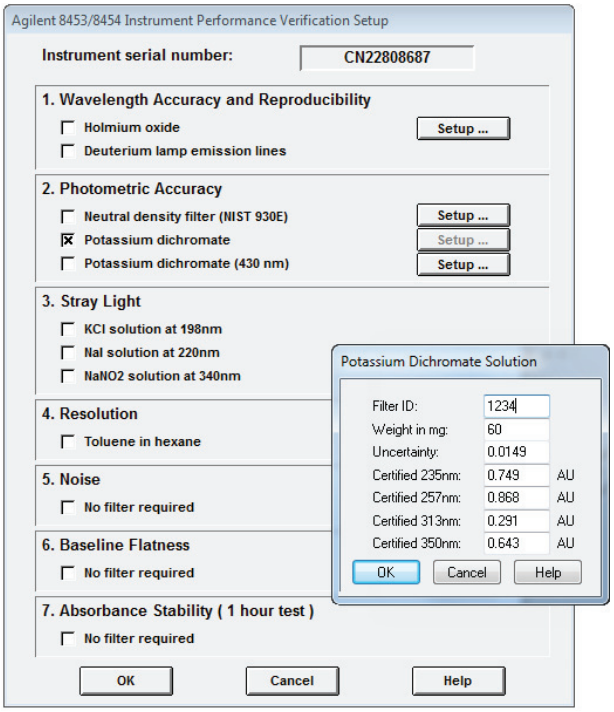

Figure 1. Instrument Performance Verification Setup menu with the new dialog for the potassium dichromate 935a photometric accuracy performance test

Uncertainty consideration

The pharmacopeia (both USP and EP) requirement is for a photometric accuracy specification of ±0.01 A. However, the CRM also has an associated uncertainty that is usually reported on the certificate. For the potassium dichromate solution this uncertainty can be as high as 0.0049 A at 60 mg/L 935a potassium dichromate. This uncertainty should be should be entered in the Uncertainty field at the start of the test.

Conclusion

The photometric accuracy specification indicates the maximum difference between the true absorbance of a sample and that measured by the spectrophotometer. While photometric accuracy can be determined using any sample which has been calibrated and has a known absorbance, in practice most modern laboratories purchase CRMs and use the certified results that are published for the CRM. For the 935a potassium dichromate photometric accuracy performance test, the UV-Visible ChemStation now uses these certified values in reporting the photometric accuracy of the instrument.

This performance test for spectrophotometers, as well as the other tests required by the pharmacopeia can be executed by the Automated Compliance Engine software available from Agilent Technologies. Using Agilent Enterprise Edition compliance services, instrument installation qualification and operation qualification is a simple process that laboratories can use to help achieve compliance with regulatory and quality requirements.

References

1. Spectrophotometry and lightscattering, *United States Pharmacopeia XXII/National Formulary XVII*, General Chapter 851, 1613

2. Absorption Spectrophotometry, Ultraviolet and Visible, *European Pharmacopoeia*, General Chapter 2.2.25, 39

3. Chris Burgess and Tom Frost UVSG (Eds), *Standards and Best Practice in Absorption Spectrometry*, Blackwell Publishing (1999), ISBN 0-632-05313-5

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