UV-Visible Spectrophotometry

Calibration of Spectrophotometer

Essentials

Regulations for quality control such as ISO 9000, Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP) or standard operating procedures in the pharmaceutical industry as required by the most important pharmacopeia's (EP, DAB, USP), require a regular performance testing of the UV/Vis spectrophotometers

Performance qualification

Performance qualification Following eight criteria should be verified with help of standard reference in calibration of uv spectrophotometer

Wavelength Accuracy
Stray Light
Resolution
Noise

Baseline Flatness
Stability
Photometric Accuracy
Linearity

Wavelength Accuracy

- Wavelength Accuracy Wavelength accuracy is defined as the deviation of the wavelength reading at an absorption band or emission band from the known wavelength of the band.

- The wavelength deviation can cause ignificant errors in the qualitative and quantitative results of the UV–Vis measurement.

It is quite obvious that if the spectrophotometer is not able to maintain an accurate wavelength scale, the UV absorption profile of the sample measured by the instrument will be inaccurate.

The true λ max and λ min of the analyte cannot be characterized accurately .

Protocol for Checking Wavelength Accuracy

Wavelength accuracy verification is checked by measuring a known wave- length reference standard with well-characterized absorption or emission peaks and comparing the recorded wavelength of the peak(s) against the value (s) listed in the certificate of that reference standard.

There are many standards that are commonly used to verify the wavelength accuracy of a spectrophotometer

- Spectra of some commonly used wavelength standards such as a deuterium lamp, mercury vapour lamp, holmium oxide filter, and holmium oxide solution (4% holmium oxide in 10% perchloric acid in a 1-cm cell).

- Acceptance. ±1 nm in the UV range (200 to 380 nm) and ±3 nm in the visible range (380 to 800 nm). Three repeated scans of the same peak should be within ±0.5nm .

Stray Light

- Stray Light Stray light is defined as the detected light of any wavelength that is outside the bandwidth of the wavelength selected.
- The causes for stray light are scattering, higher-order diffraction of the monochromator , or poor instrument design.
- Stray light causes a decrease in absorbance and reduces the linear range of the instrument.
- High-absorbance measurements are affected more severely by stray light .
- The stray light problem causes a deviation from linearity at high absorbance

PROTOCOL

For the stray light test, various cut-off filters or solutions can be used to estimate the stray light contribution, depending on the wavelengths is used.

Scan the stray light testing solution in a 1-cm cell using air as the reference.

The absorption of a solution of potassium chloride R (12 g/l) between 220 nm and 200 nm in a light path of 1 cm must rise steeply and at a wavelength of 198 nm must be greater than 2, measured against water R as a compensation liquid

Resolution

- Resolution The resolution of a UV-Vis spectrophotometer is related to its spectral bandwidth (SBW). The smaller bandwidth, the finer the resolution. The SBW depends on the slit width and the dispersive power of the monochrometer . Typically, only spectrophotometers designed for high-resolution work have a variable slit width.

Spectrophotometers for routine analysis usually have a fixed slit width.

The resolution of the absorbance measurement depends on the ratio of the spectral bandwidth (SBW) of the spectrophotometer to the natural bandwidth (NBW) of the spectral band to be measured.

PROTOCOL

The resolution of the absorbance measurement depends on the ratio of the spectral bandwidth (SBW) of the spectrophotometer to the natural bandwidth (NBW) of the spectral band to be measured

Acceptance : The ratio of the absorbance at λ_{max} (269 nm) and absorbance at λ_{min} (266 nm) should be greater than 1.5.

Noise

Noise in the UV-Vis measurement originates primarily from the light source and electronic components.

Noise in the measurement affects the accuracy at both ends of the absorbance scale.

Photon noise from the light source affects the accuracy of the measurements at low absorbance.

- Electronic noise from the electronic components affects the accuracy of the measurements at high absorbance.

- A high noise level affects the precision of the measurements and reduces the limit of detection, thereby rendering the instrument less sensitive.

PROTOCOL

Air is scanned in the absorbance mode for 10 min. peak-to-peak noise is recorded at 500 nm.

The root mean square (RMS) noise is then calculated. Modern spectrophotometers are usually equipped with the noise estimation function.

Acceptance: The RMS noise should typically be less than 0.001 AU

Baseline Flatness

Baseline Flatness The intensity of radiation coming from the light source varies over the entire UV-Vis range. Most UV-Vis spectrophotometers have dual light sources.

A deuterium lamp is used for the UV range and a tungsten lamp is used for the visible range. The response of the detector also varies over the spectral range.

The flat baseline test demonstrates the ability of the instrument to normalize the light intensity measurement and the spectral output at different wavelengths throughout the spectral range .

PROTOCOL

Air is scanned in the absorbance mode. The highest and lowest deflections in the absorbance unit are recorded.

Acceptance: The deflection is typically less than 0.01 AU.

Stability

Stability Variations in lamp intensity and electronic output between the measurements of the reference and the sample result in instrument drift.

The lamp intensity is a function of the age of the lamp, temperature fluctuation, and wavelength of the measurement.

These changes can lead to errors in the value of the measurements, especially over an extended period of time.

The resulting error in the measurement may be positive or negative.

The stability test checks the ability of the instrument to maintain a steady state over time so that the effect of the drift on the accuracy of the measurements is insignificant.

PROTOCOL

Air is scanned in the absorbance mode for 60 min at a specific wavelength (typically, 340 nm). The highest and lowest deflections in the absorbance unit are recorded.

Acceptance: The deflection is typically less than 0.002 AU/h .

Photometric Accuracy

Photometric accuracy is determined by comparing the difference between the measured absorbance of the reference standard materials and the established standard value.

An optically neutral material with little wavelength dependency for its transmittance/absorbance is desirable because it eliminates the spectral bandwidth dependency of measurements.

PROTOCOL

In that 0.006 % w/v solution of potassium dichromate (60.06 mg/L) is prepared in 0.005 M sulphuric acid. A 0.005 M sulphuric acid solution as the reference is scanned and then the potassium dichromate solutions are scanned at 235, 257, 313, and 350 nm.

Acceptance : Six replicate measurements of the 0.006% w/v potassium dichromate solution at 235, 257, 313, and 350 nm should be less than 0.5% RSD.

Linearity

Linearity The linear dynamic range of the measurement is limited by stray light at high absorbance and by noise at low absorbance.

For routine measurements involving samples and the related reference chemical standards, the accuracy of the quantification of the sample depends on the precision and linearity of the measurements

PROTOCOL

A series of potassium dichromate solutions of concentration 20, 40, 60, 80, and 100 mg/L in 0.005 M sulphuric acid can be used to test the linearity of the system.

First, 0.005 M sulphuric acid as reference is scanned and then the potassium dichromate solutions at 235, 257, 313, and 350 nm.

- The absorbance values at various wavelengths are plotted against the concentration of the solutions and the correlation coefficients are calculated.

- Acceptance: Correlation coefficient $r = 0.999$

Materials used as Standard

Ideally used type of filters

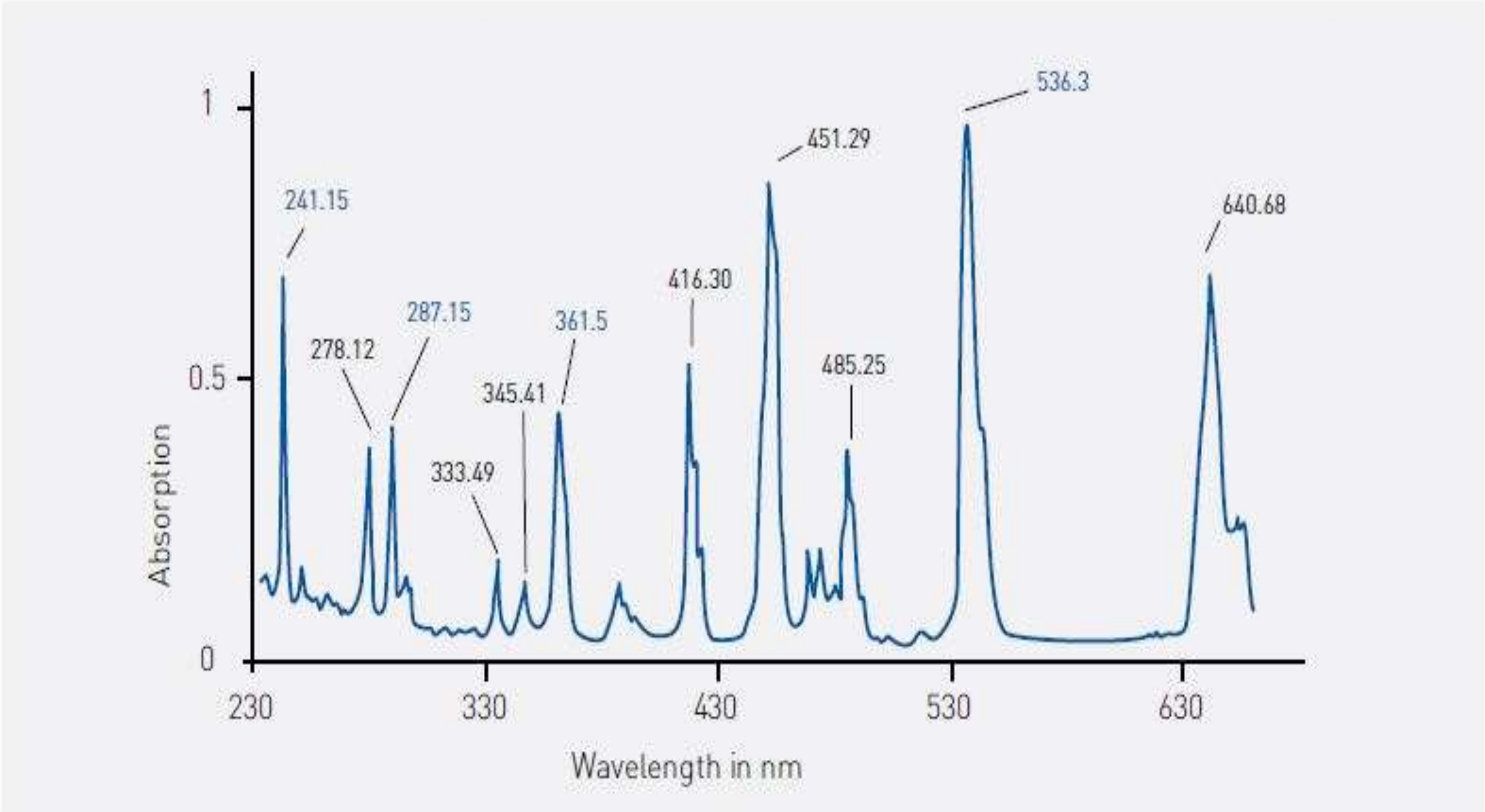
Usually by trial and error following type of filters are considered ideal for working with all kinds of spectrophotometric instruments.

For checking Wavelength accuracy, Photometric accuracy, Stray light behaviour & Resolution Liquid filters are more reliable whereas for visible ranges solid filters are preferred to calibrate the instrument for Wavelength and Photometric accuracy

Holmium oxide liquid filter (Holmium oxide dissolved in perchloric acid)

- The exact position of peaksat 241.15 nm, 287.15 nm,361.5 nm and 536.3 nm is determined and compared with the values in the certificate.

- Deviations should not be greater than ± 1 nm in the ultra-violet and ± 3 nm in the visible range



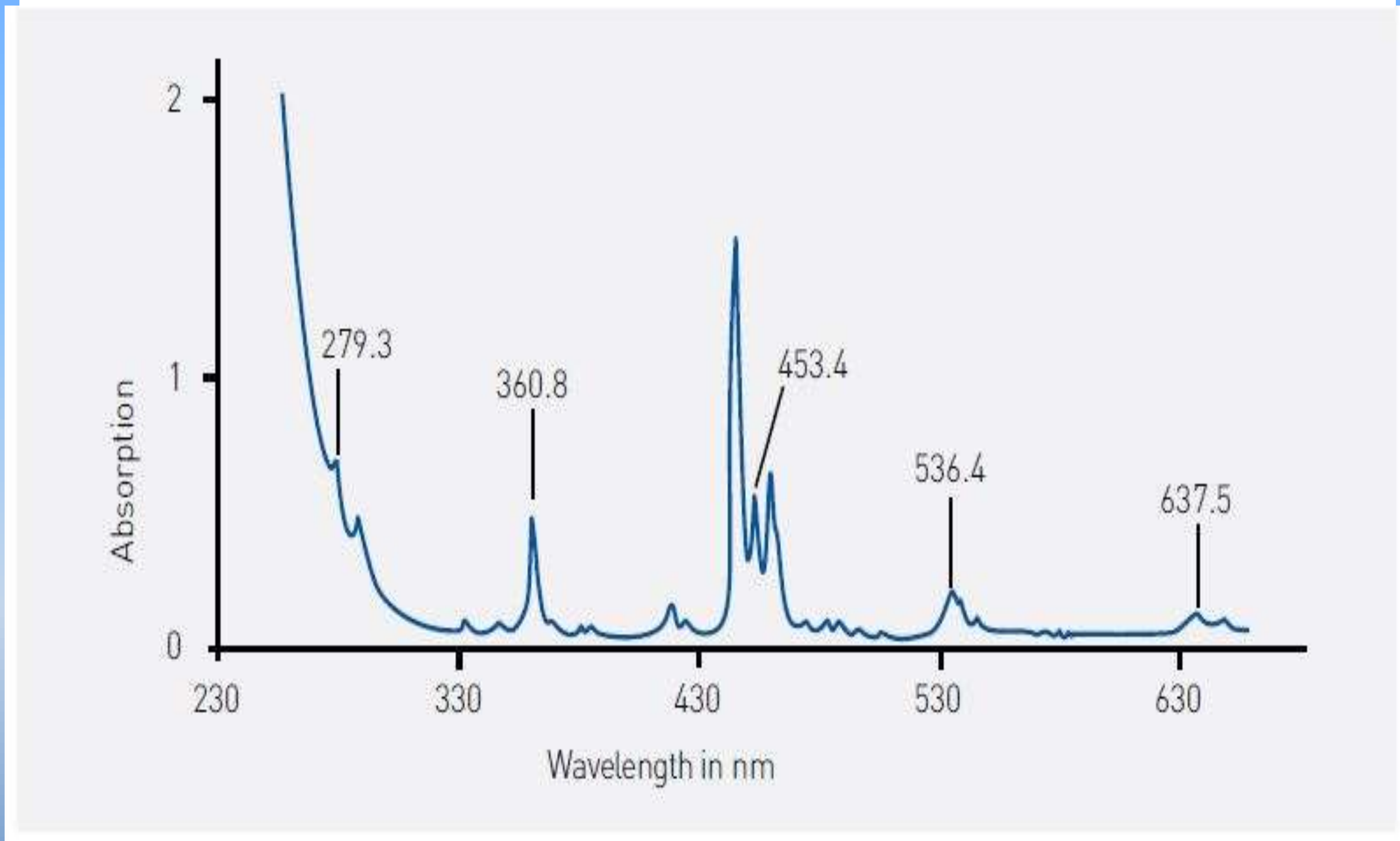
Holmium Oxide Spectra

Theoretical Wavelength according to Pharm. Eur.	241.15 nm 287.15 nm 361.5 nm 536.3 nm

Slit width= 1nm (or to be kept as low as possible)

Testing the wavelength accuracy in the UV and visible range between 240 nm and 650 nm for spectrophotometers with a spectral bandwidth (slit) between 0.1 nm and 3 nm

Wavelength at	279.3 nm 360.8 nm 453.4 nm 536.4 nm 637.5 nm

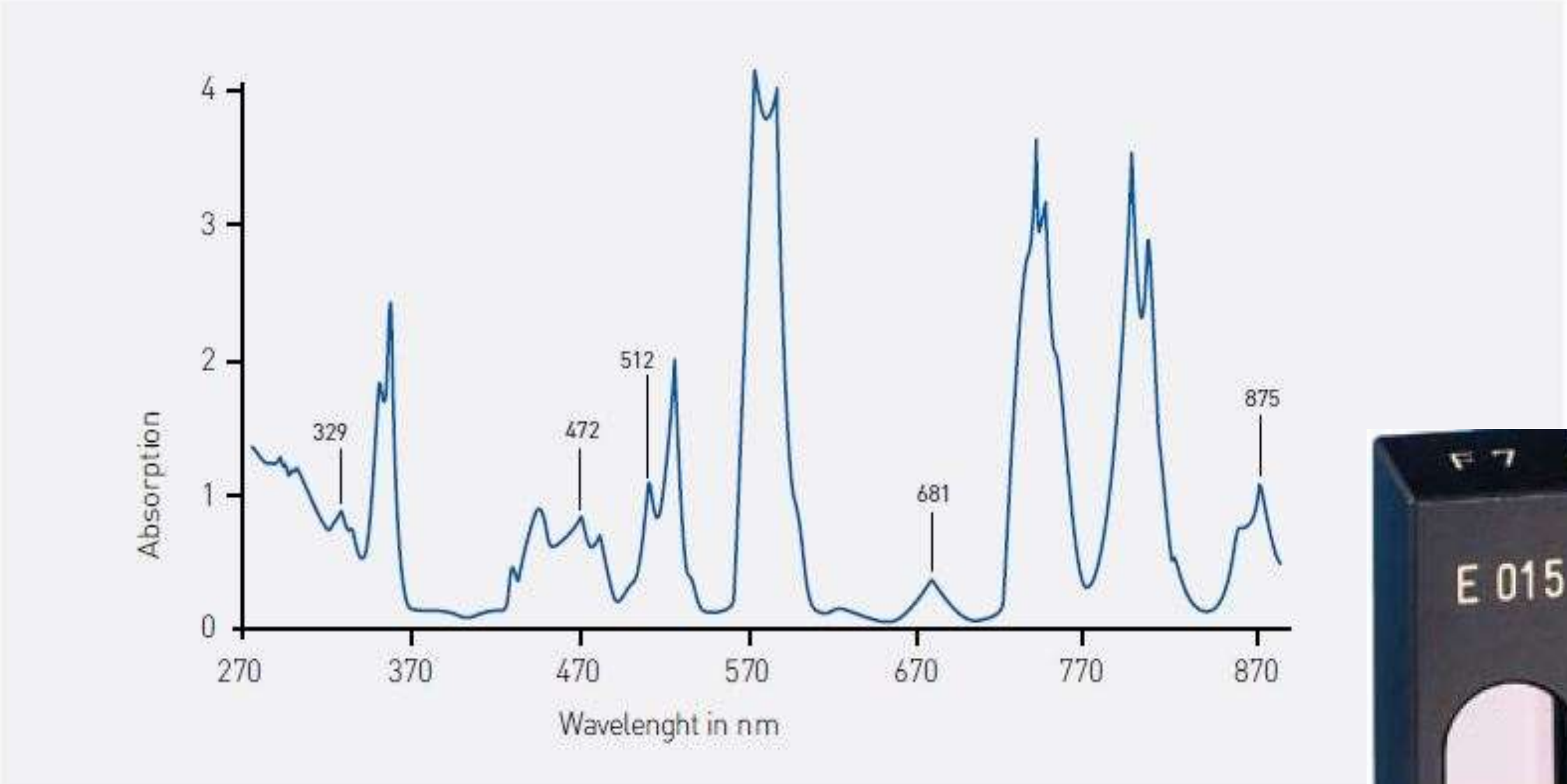


Holmium Oxide Glass doped Filter and its spectra
(2.1 mm thickness)

Didymium oxide

- Generally available as glass filter is more reliable for routine calibration made out of special grade glass.

- Testing the wavelength accuracy in the UV and Vis region (329 nm to 875 nm) at a spectral bandwidth to 2 nm.



Spectra of Didymium Glass Filter (spectral bandwidth to 2 nm)

Absorbs at-

329 nm

472 nm

512 nm

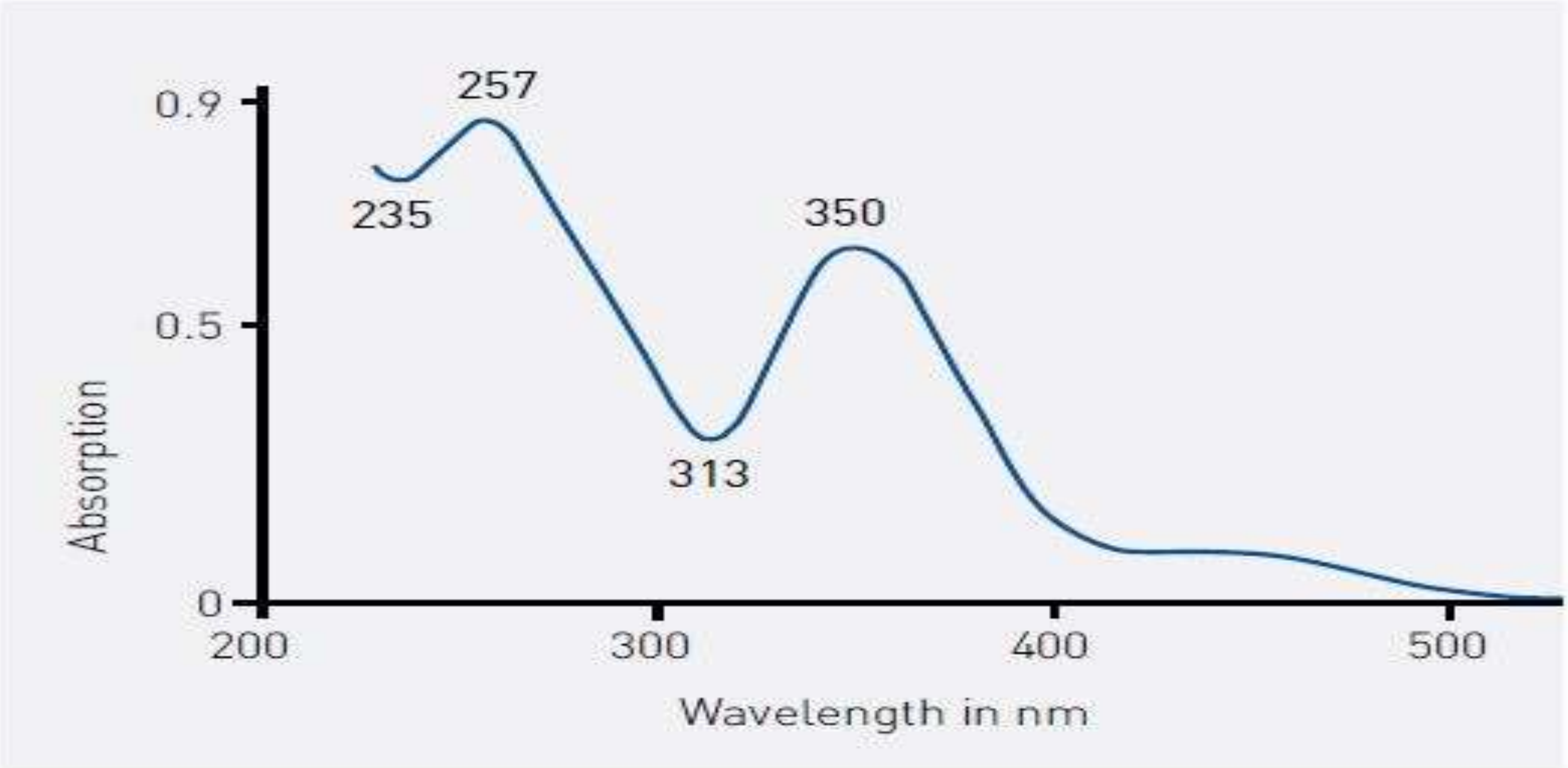
681 nm

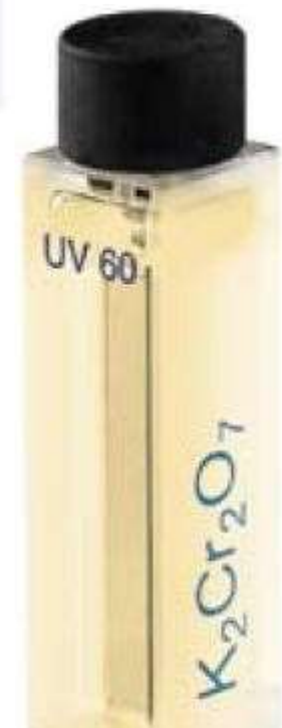
875 nm

Potassium Dichromate

Potassium dichromate dissolved in perchloric acid is suitable for testing the photometric accuracy (absorbance) of spectrophotometers in the UV spectrum.

The absorption values of the cuvette are measured at the wavelengths 235 nm, 257 nm, 313 nm and 350 nm and recorded in the certificate

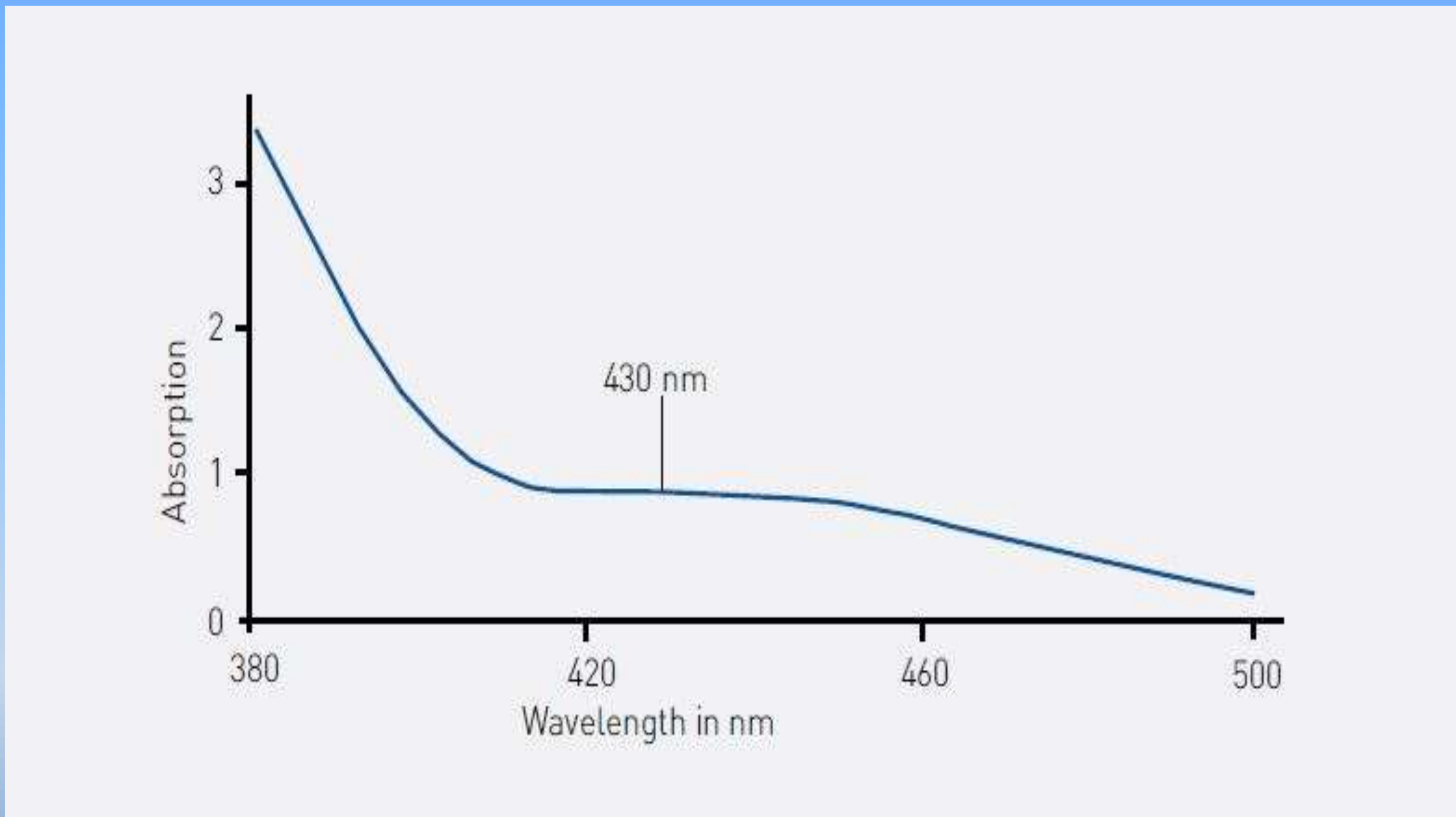




Typical spectrum of a 0.006 % potassium dichromate solution (spectral bandwidth of 2 nm)

Absorbs at
235 nm
257 nm
313 nm
350 nm

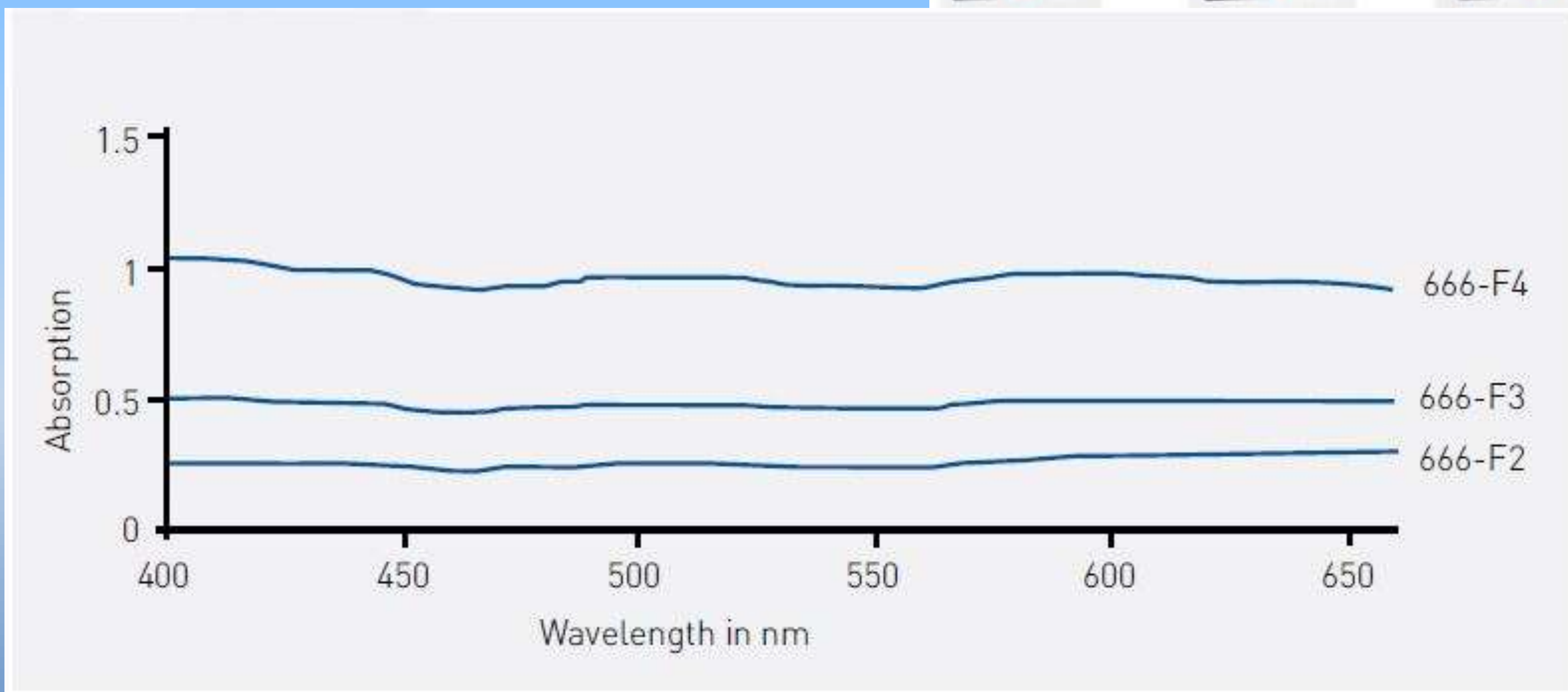
Potassium dichromate liquid filter (430 nm)



Typical spectrum of a 0.06 % potassium dichromate solution

Neutral density glass filter

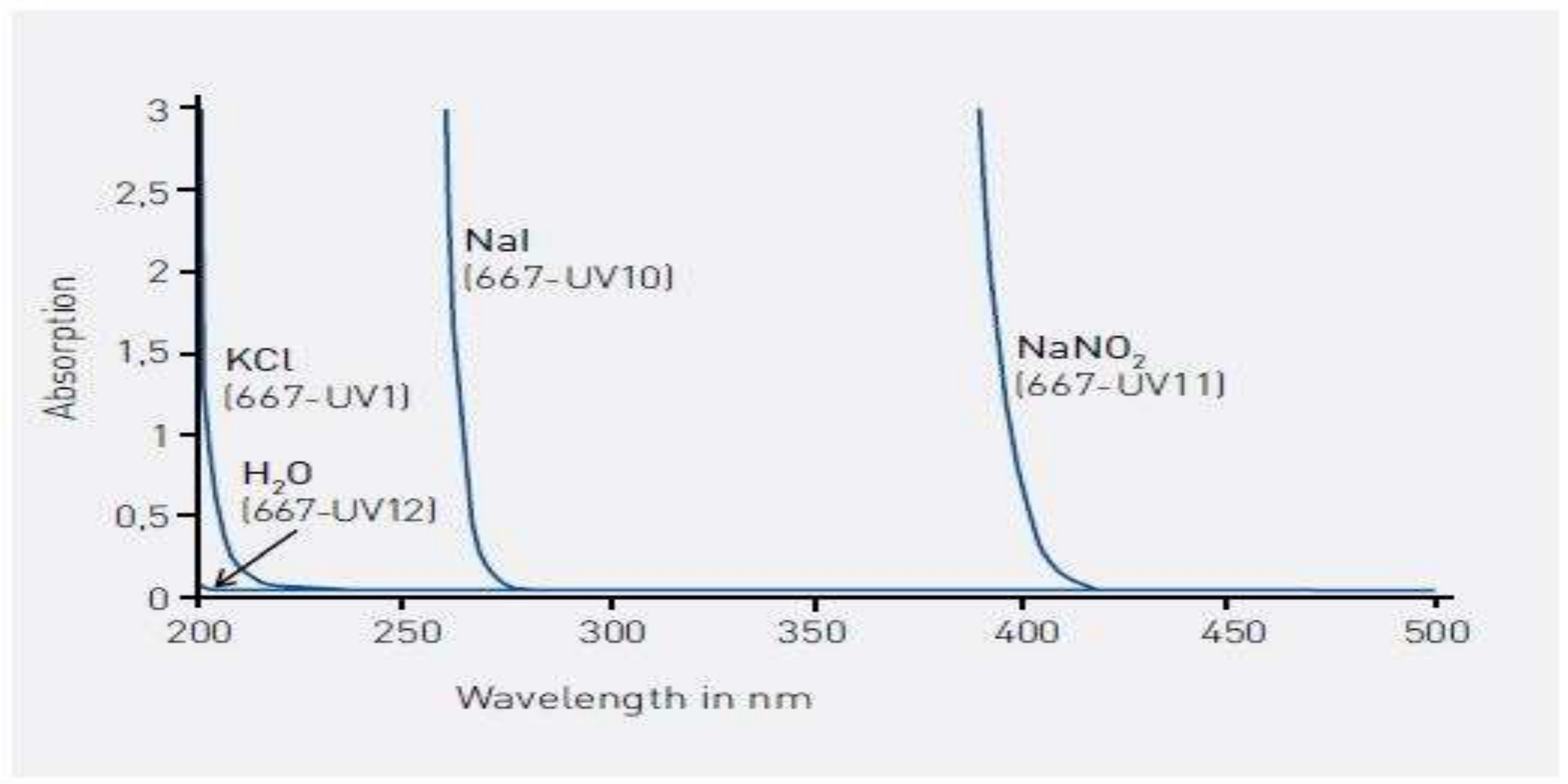
Absorbs in visible region (440 nm to 635 nm)
Made of SCHOTT Glasss



Typical spectrum of the neutral density glass filter, taken with a slit width of 1 nm

Cut-Off filters

Spectra of various cut-off filters
Testing for stray light in the UV spectrum (at wavelengths from 198 nm to 385 nm, depending on the selected filter)



Application

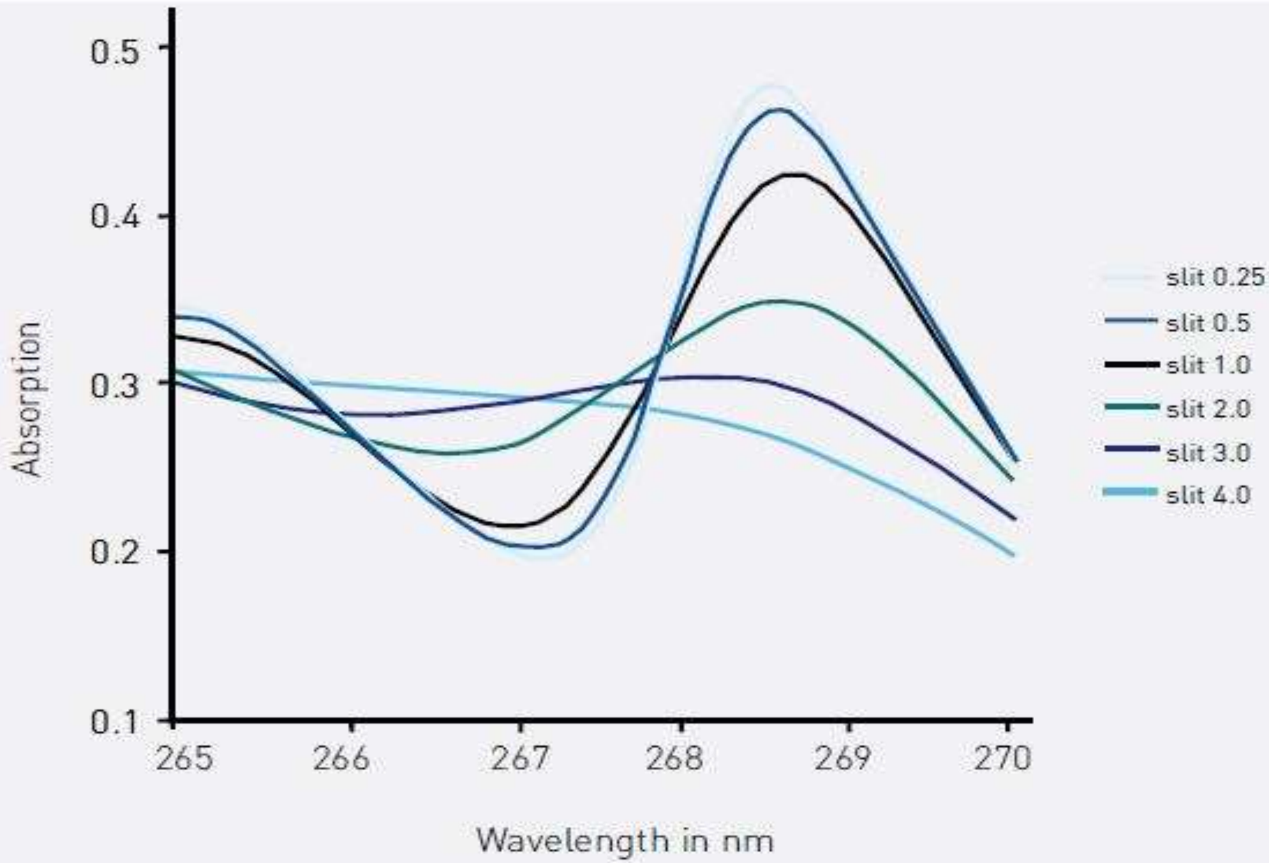
The photometer is set to a starting wavelength that is 30 nm above the cut-off wavelength of the filter to be used. If, for example, potassium chloride is used then the start is at 230 nm.

Then a scan is carried out down towards shorter wavelengths and the absorption below the cut-off wavelength is observed. The transmission that is measured below the cut-off wavelength is stray light

Calibration standards for testing the resolution

The resolution for modern photometers depends directly on the slit width. The smaller the slit and the associated spectral bandwidth, the higher the resolution.

The choice of material should be made as per the standards applicable to the analysis or as mentioned on the format for certificate of analysis or the manual.



Typical spectra of the Toluene liquid filters with varying slit widths

In order to determine the resolution of a spectrophotometer, the behaviour of the absorption of a maximum at λ_{max} (269 nm) to absorption of a minimum at λ_{min} (266 nm) is shown.

From this, one obtains details of the spectral bandwidth of the instrument used. Wavelength spectrum from 265 nm to 270 nm is scanned and the peak maximum and peak minimum are determined. Then the relationship between the two is calculated

Two type of standards are used-

1. Toluene in n-Hexane
2. Hexane as reference

Slit width in nm	Ratio (Recommended values)
0.25	2.3
0.5	2.2
1.0	2.0
2.0	1.4
3.0	1.1
4.0	1.0

Commonly used methods

Control of Absorbance (Potassium Dichromate method)

- Dry a quantity of Potassium Dichromate by heating to constant weight at 130^0C .

- Weigh and transfer accurately a quantity not less than 57.0mg and not more than 63 mg to 10000ml volumetric flask. Dissolve and dilute in sufficient 0.005M H_2SO_4 to produce 1000ml.

- Measure the absorbance of Potassium Dichromate solution at wavelength given in the table

- Calculate value of $\{A^{1\%}_{1cm}\}$ for each wavelengths mentioned.

- Acceptance Criteria: (as per I.P and B.P)

Wavelengths(in nm)	A(1%/1cm)	Limit
235.0	124.5	129.9-126.2
257.0	144.0	142.8-145.7
313.0	48.6	47.0-50.3
350.0	106.6	105.6-108.2

Resolution power

- Record the spectrum of a 0.02% v/v solution of toluene in hexane in the range of 260nm to 420 nm (before use check the hexane for transmittance, using water as blank between 260nm to 420 nm & use only if transmittance is not less than 97%)

- Acceptance Criteria: The ratio of absorbance at maximum at about 269 nm to that at the minimum at about 266 nm is not less than 1.5

Limit of Stray Light

- Prepare a 1.2% w/v solution of potassium chloride in water.
- Measure the absorbance of the above solution at solution at 198.0, 199.0, 200.0, 201.0 and 202.0 using water as blank.
- Acceptance Criteria:

  Absorbance is greater than 2

Wavelength accuracy, Resolution & baseline flatness

- These are instruments in-built functions
- The note made here is in accordance to Shimadzu UV-1700 UV-Vis Spectrophotometer
- Attach printer directly to the instrument (optional)
- Goto MODE and press F3 key to opt for maintenance mode.
- Press 1

(…continued)

- Press Start/Stop key.
- After screen changes, ensure that nothing is kept in the optical path & press the start/stop key again.
- Prints will come after all the tests are over.
- Acceptance Criteria:

For wavelength accuracy: at 656.1±0.3 nm & at 486.0±0.3 nm.

Resolution:1.0 nm or less.

Baseline flatness: ±0.002Abs.

Calculation :

$$A_{1\,cm}^{1\%} = \frac{\text{Absorbance} \times 10000}{\text{Weight of potassium dichromate in mg}}$$

Reference

- #2 U.S. Pharmacopoeia
- #3 Pharmaguideline
- #4 Hellma GmBH product Catalogue
- #5 Schimadzu UV-1700 Users manual


That was
boooooringgggg!!!