



Calibration Of UV Visible spectrophotometer



WHAT IS CALIBRATION

- Calibration is the act of ensuring that a method or instrument used in measurement will produce **accurate results.**
 - Calibration is performed using **primary reference standard**
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NEED OF CALIBRATION

- When a specific **time period** is elapsed.
 - When a specific operating hours has elapsed.
 - With a new instrument.
 - When observations appear questionable.
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UV APPARATUS



SHIMADZU UV 1601

SHIMADZU UV 2600






Perkin Lambda 650



Labindia 3092

PROCEDURE OF CALIBRATION

1. Control of wavelength
 2. Control of absorbance
 - For ultraviolet region
 - For visible region
 - Limit of stray light
 - resolution power
 3. Maintenance
 4. Precautions
 5. Abbreviations
- 

CONTROL OF WAVELENGTH

CHEMICALS: 1.4M
perchloric acid,
holmium
perchlorate
solution



STEP 1: Dissolve 1
gram of holmium
oxide in 1.4M
perchloric acid
with the aid of
heating on water
bath, cool and
dilute to 25ml with
same solvent.



STEP 2: Record the
spectrum holmium
perchlorate
solution from
200nm to 600nm
using 1.4M
perchloric acid as
reference solution

- Note down the maxima observed at wavelength against the acceptance criteria given below:

S.No.	Maxima Wavelength (nm)	Tolerance (nm)
1.	241.15nm	240.15nm to 242.15nm
2.	287.15nm	286.15nm to 288.15nm
3.	361.5nm	360.50nm to 362.50nm
4.	536.3nm	533.30nm to 539.30nm

WAVELENGTH ACCURACY

- Deviation of wavelength reading at an absorbance band

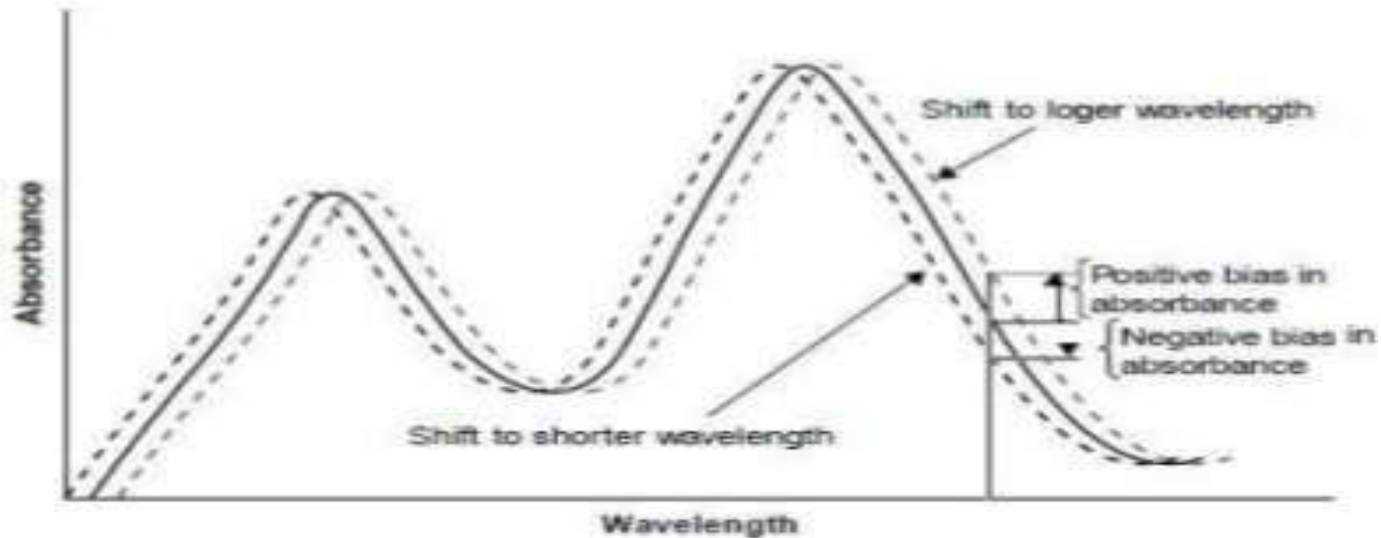


Figure 10.2. Effect of wavelength accuracy on UV-Vis measurements.

CONTROL OF ABSORBANCE

STEP 1: Weigh 57 to 63mg of potassium dichromate primary standard and transfer to 1000ml volumetric flask. Dissolve in 0.005M sulphuric acid and make up to the mark with the same acid.

Step 2: Measure the absorbance at 235 nm, 257nm, 313nm and 350nm using 0.005M sulphuric acid as reference

- **FOR VISIBLE REGION:** Whole procedure is same as UV region but at the end measure the absorbance at 430nm.
- **CALCULATION:** Value of A(1%,1cm)

$$A(1\%,1\text{cm}) = \frac{\text{absorbance} \times 100}{\text{weight in gm} \times 100}$$

Table I

S.No.	Wavelength (nm)	Absorbance E (1%1cm)	Maximum tolerance
1.	235	124.5	122.9 to 126.2
2.	257	144.0	142.8 to 145.7
3.	313	48.6	47.0 to 50.3
4.	350	106.6	104.9 to 108.2
5.	430	15.9	15.7 to 16.1

LIMIT OF STRAY LIGHT

STEP 1: Prepare the solution of 1.2%v/v potassium chloride and dissolve with 50ml distilled water.



STEP 2: Determine the absorbance using path length of 1cm at 200nm against purified water as blank



Acceptance Criteria: Absorbance at 200nm should be Greater than 2.0

RESOLUTION POWER

STEP 1: Prepare a solution 0.02%v/v toluene in hexane


STEP 2: Record the spectrum of 0.02%v/v toluene in hexane from 250nm – 300nm using hexane as reference

STEP 3: record the absorbance at 269nm (max) and 266nm (min)


STEP 4: Calculate the ration of absorbance by dividing the absorbance at maxima and minima

ACCEPTANCE CRITERIA: Absorbance ratio at 269nm to 266nm is not less than 1.5

MAINTENANCE

- When the instrument not produce calibrated results it should be labeled “FAUL TY” and should be repaired by service engineer of instrument.
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PRECAUTIONS

- The instrument should be always free from dust
 - Place the instrument as far as possible from any strong magnetic or electric field or any electric apparatus generating a high frequency.
 - Avoid places where vibrations exist or direct sunlight.
 - To extent the life of source lamp have them powered only when required.
 - The temp. Of all solution used in the test should not differ by more then 0.5°C .
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CALIBRATION OF INSTRUMENT

Clause No.: 5.5F-03

Name of Instrument	UV-Visible Spectrophotometer	Instrument No.	DIVLERI-006
Make	Shimadzu	Calibrated on	29-09-2017
Model No.	1800	Calibration Due on	28-03-2018
Location	Air Section	SOP Ref	DRL-QC-081

1. CONTROL OF WAVELENGTH (BY HOLMIUM PERCHLORATE SOLUTION):

MAXIMA FOUND AT	STANDARD WAVELENGTH	TOLERANCE LIMIT
241.00	241.15 nm	± 1 nm (200 nm – 400 nm)
287.00	287.15 nm	± 1 nm (200 nm – 400 nm)
361.50	361.50 nm	± 1 nm (200 nm – 400 nm)
526.50	536.30 nm	± 3 nm (400 nm – 600 nm)

Remarks: The instrument is calibrated / not-calibrated and found satisfactory for work.

Calibrated by *[Signature]*
Date 29/09/2017

Approved by *[Signature]*
Date 29/09/2017

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DTRL/QA/F-48

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ISSUED BY: *[Signature]*

ISSUED DATE: 29-09-2017



CALIBRATION OF INSTRUMENT

		Clause No. 5.5F-03	
Name of Instrument	UV-Visible Spectrophotometer	Instrument No	DTRL-EQ1-006
Make	Shimadzu	Calibrated on	29-09-2017
Model No	1800	Calibration Due on	28-02-2018
Location	Riv Section	SOP Ref	DTRL-BCC-081

1. LIMIT OF STRAY LIGHT:

Solution used	Wavelength	Absorbance Observed*	Absorbance Limit	Remark
1.2 % w/v solution of KCl in water	200 nm	2.204	NLT 2.0	OK.

*Absorbance of a 1.2 w/v solution of potassium chloride in a 1 cm cell at about 200nm when compared with water as reference liquid.

2. RESOLUTION POWER:

Solution used	Sr.	Wavelength	Absorbance	Ratio (A/B)	Limit	Remark
0.02 % v/v solution of toluene in hexane	A	269 nm	0.341	1.55	NLT 1.5	OK.
	B	266 nm	0.220			

3. CONTROL OF ABSORBANCE:

For wavelength 235, 257, 313 & 350 nm:

Solution prepare by using 57.0 to 63.0 mg Potassium dichromate in 1000 ml of 0.005M Sulphuric acid.

Wt. of potassium dichromate = 5.9618 gm

For wavelength 430 nm:

Solution prepare by using 57.0 to 63.0 mg Potassium dichromate in 100 ml of 0.005M Sulphuric acid.

Wt. of potassium dichromate = 0.0601 gm

Sr. No.	Wavelength (nm)	Absorbance A (1%, 1cm)	Absorbance Calculation [Absorbance (A)/Conc.]	Result	Maximum Tolerance
1.	235	0.762	122.46	OK	122.9 to 126.2
2.	257	0.295	144.82	OK	142.8 to 146.2
3.	313	0.295	47.73	OK	47.0 to 50.3
4.	350	0.661	106.96	OK	105.6 to 109.0
5.	430	0.958	15.94	OK	15.7 to 16.1

Remarks: The instruments is calibrated / not-calibrated and found satisfactory for work.

Calibrated by *[Signature]*
Date 29/09/17

Approved by *[Signature]*
Date 29/09/2017

DTRL/QP/F-49

ISSUED BY: *[Signature]*
ISSUED DATE: 29-09-2017

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A close-up photograph of a man in a dark suit, white shirt, and patterned tie. He is holding a silver pen in his right hand, writing the words "Thank you" in a white, elegant cursive script on a dark surface. His left hand is partially visible at the bottom right, with fingers slightly curled. The lighting is dramatic, highlighting the man's hands and the pen against the dark background.

Thank
you