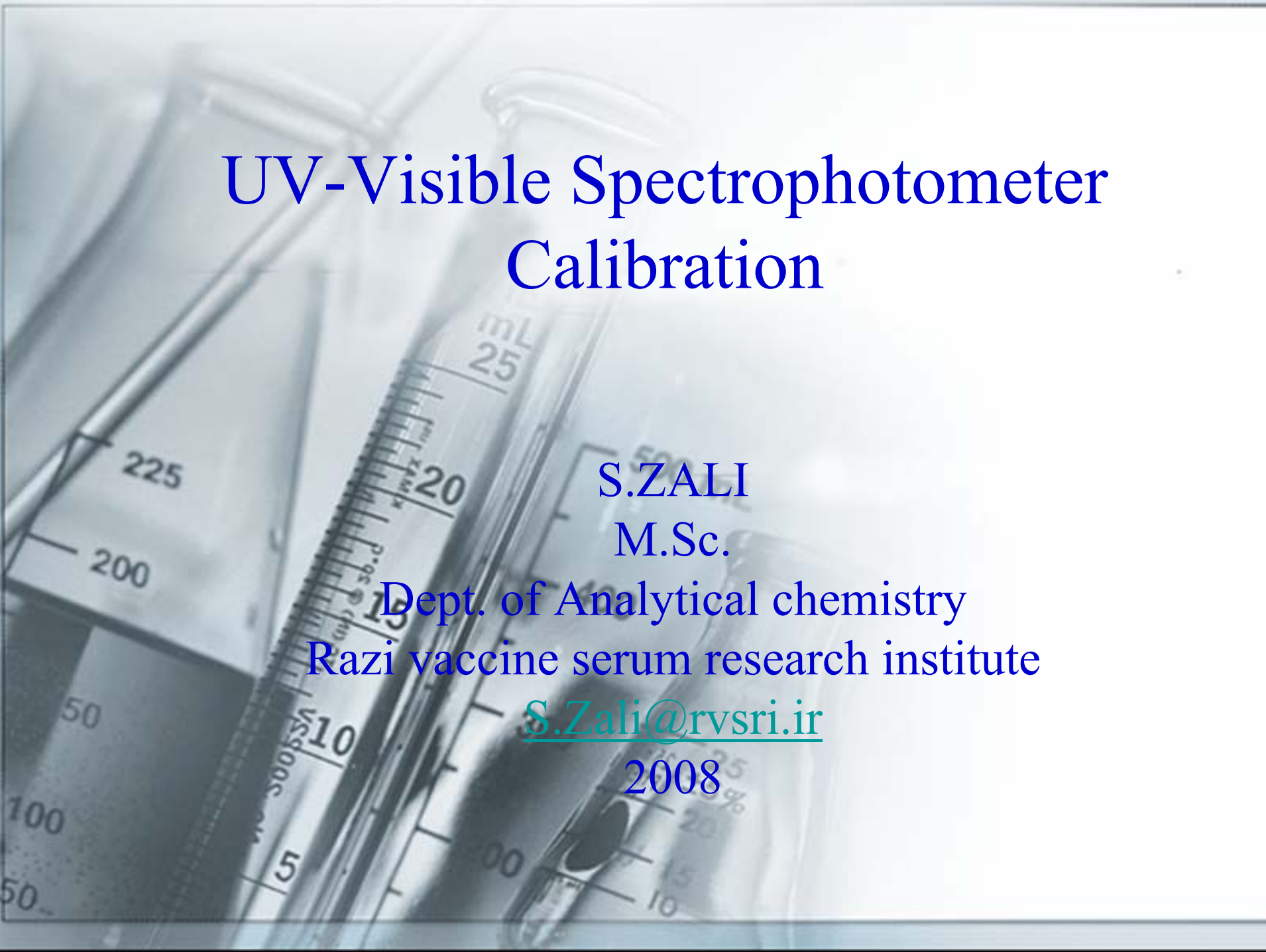




# UV-Visible Spectrophotometer Calibration

S.ZALI  
M.Sc.

Dept. of Analytical chemistry  
Razi vaccine serum research institute

[S.Zali@rvsri.ir](mailto:S.Zali@rvsri.ir)

2008

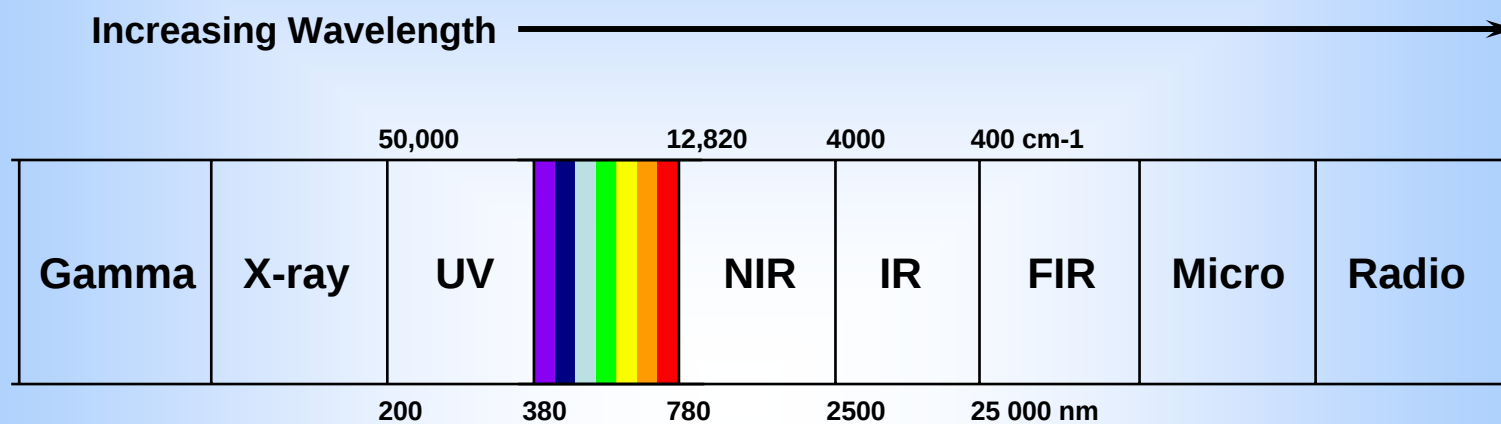


The primary objective of GLP is to ensure the generation of high quality data. Essential to this is the ability to produce estimates for the measurement uncertainty associated with calibrations.

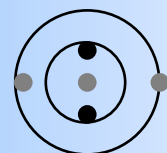
The spectrophotometer has become the workhorse for high throughput routine testing for a wide variety of analysts in chemical and biochemical analysis

laboratories. It is important for accuracy and reproducibility in measurement that this instrument is correctly set up and calibrated.

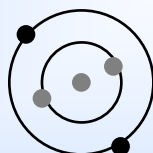




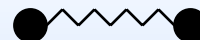
← Increasing Energy



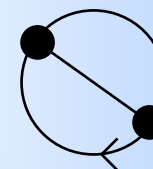
K-shell  
electrons



Outer-shell  
electrons

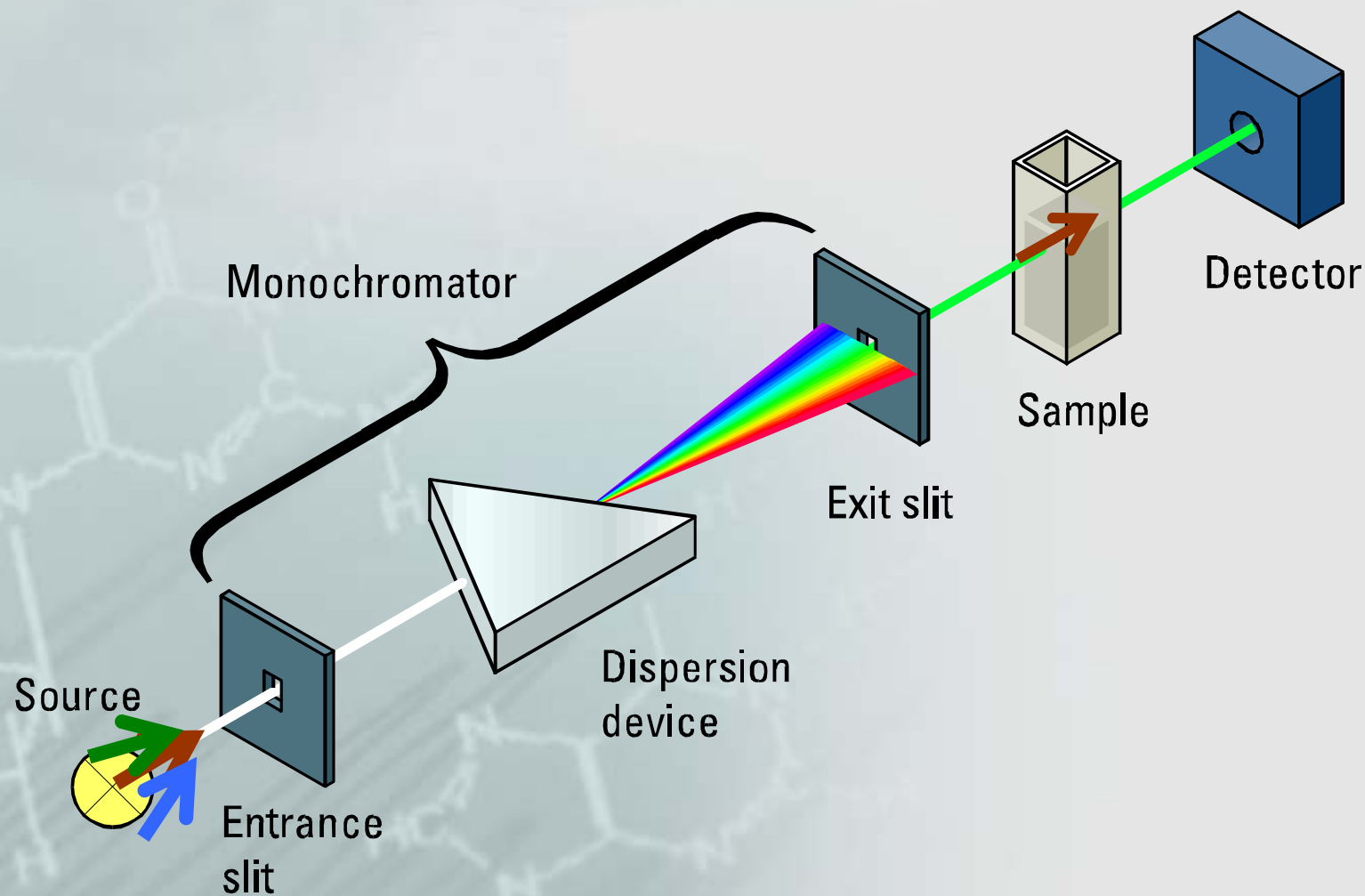


Molecular  
vibrations



Molecular  
Rotation

# Conventional Spectrophotometer:



# The factors that must be calibrated:

Control of wavelengths

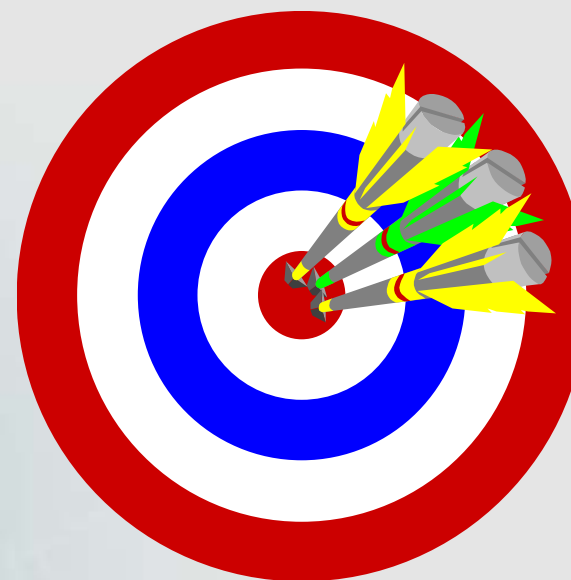
Control of absorbance

Limit of stray light

Resolution

Spectral slit width

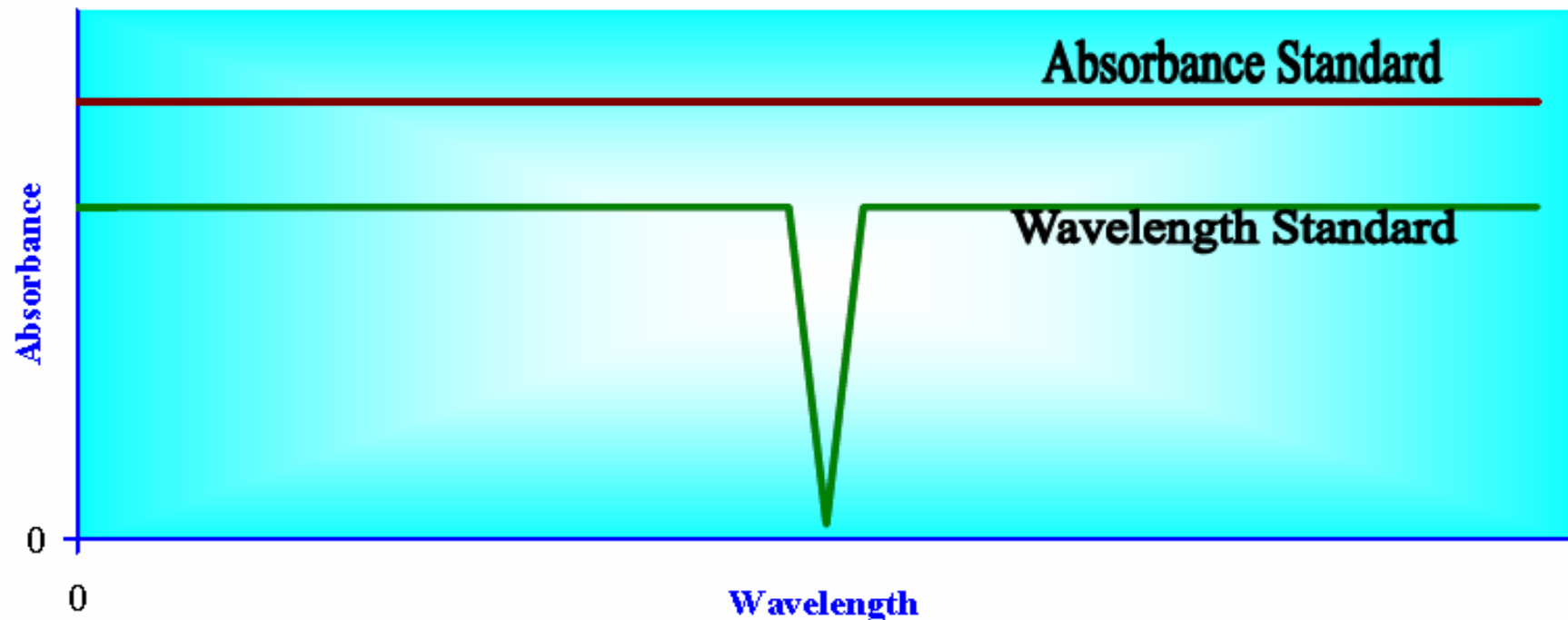
Cell



# Control of wavelengths:

## Wavelength standards:

The ideal wavelength standard would have very narrow and very well defined peaks.



# Control of wavelengths:

- Solution of holmium(III) in perchloric acid: 240–640 nm.
- Holmium oxide filters: 280–640 nm.
- Didymium filters: 400–750 nm.
- Using of spectral lines in low-pressure discharge lamps: 656.1 nm produced by the D2 lamp.

# Holmium(III) in perchloric acid:

Consists of a 4% W/V solution of Holmium Oxide in 1.4M Perchloric Acid.

$\lambda_{\text{max}}$ ..... $241.1 \pm 0.4\text{nm}$

$278.0 \pm 0.4\text{nm}$

$287.5 \pm 0.4\text{nm}$

$361.2 \pm 0.4\text{nm}$

$416.6 \pm 0.4\text{nm}$

$451.3 \pm 0.4\text{nm}$

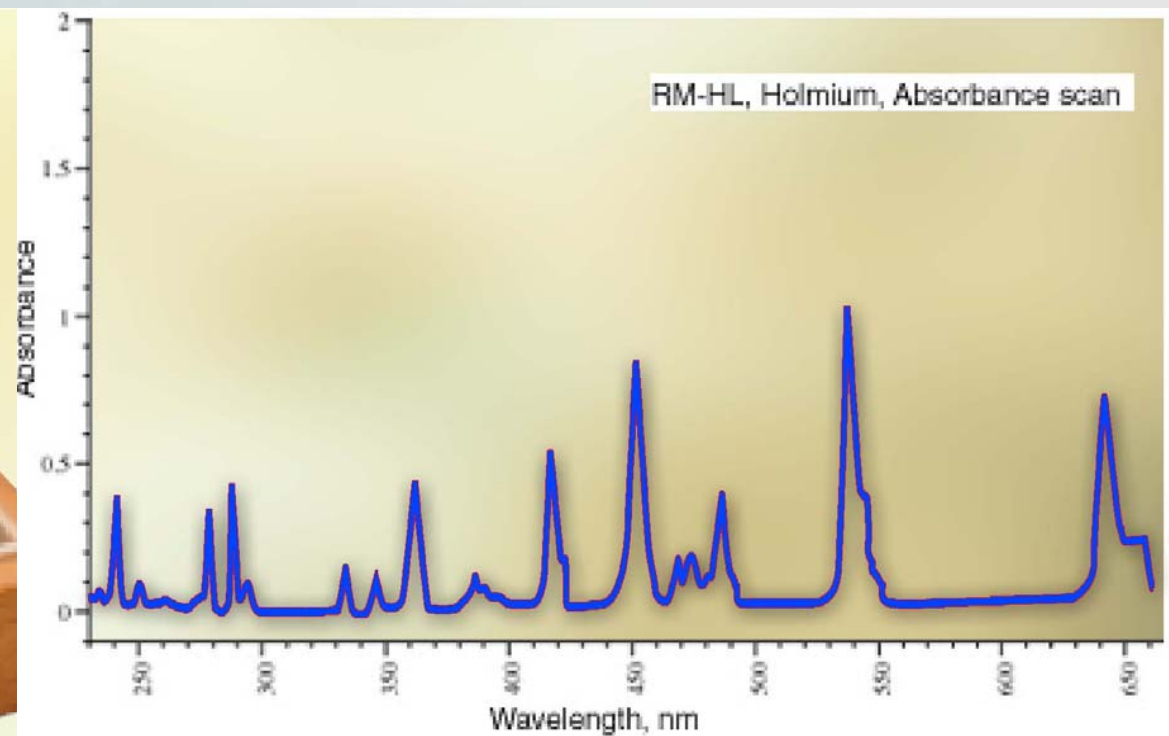
$458.3 \pm 0.4\text{nm}$

$537.5 \pm 0.4\text{nm}$

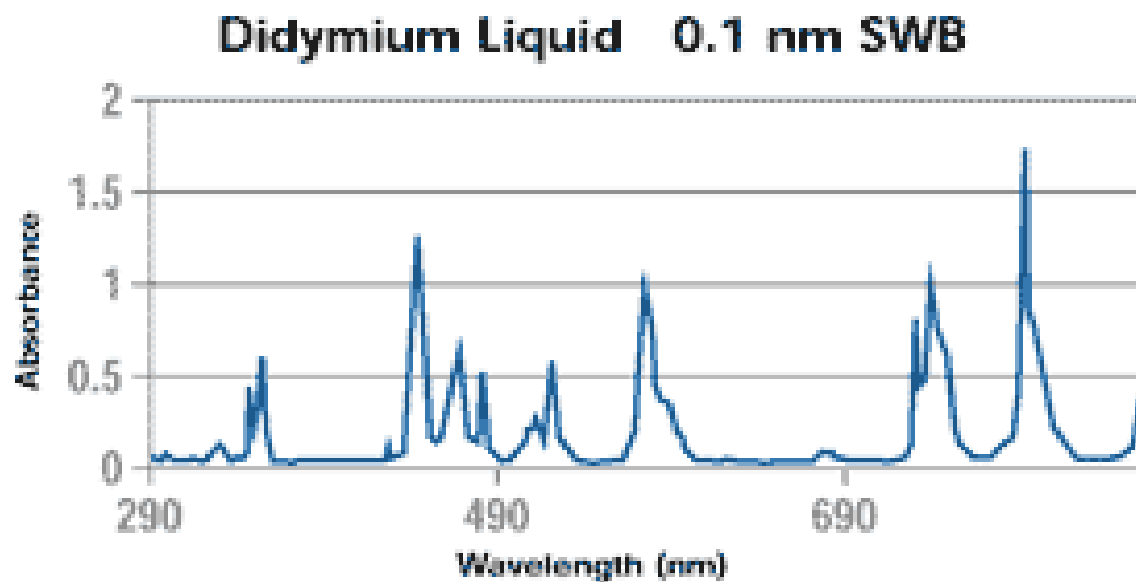
$640.8 \pm 0.4\text{nm}$



# Holmium oxide filters:



# Didymium filters:



# Discharge lamps:

A quick check of the wavelength accuracy of a spectrophotometer can be performed by observing the strong emission band at 656.1 nm or 486.0 nm produced by the D2 lamp.

In fact many of modern, so-called self calibrating instruments make use of deuterium emission lines to set the wavelength scales.



# British pharmacopoeia 2007:

Table 2.2.25.-1. – *Absorption maxima for control of wavelength scale*

|                |                       |
|----------------|-----------------------|
| 241.15 nm (Ho) | 404.66 nm (Hg)        |
| 253.7 nm (Hg)  | 435.83 nm (Hg)        |
| 287.15 nm (Ho) | 486.0 nm (D $\beta$ ) |
| 302.25 nm (Hg) | 486.1 nm (H $\beta$ ) |
| 313.16 nm (Hg) | 536.3 nm (Ho)         |
| 334.15 nm (Hg) | 546.07 nm (Hg)        |
| 361.5 nm (Ho)  | 576.96 nm (Hg)        |
| 365.48 nm (Hg) | 579.07 nm (Hg)        |



- The permitted tolerance is  $\pm 1$  nm for the ultraviolet range and  $\pm 3$  nm for the visible range. Suitable certified reference materials may also be used.

# United States pharmacopoeia:

The 486.13 - nm and 656.28-nm lines of a hydrogen discharge lamp.

Suitable glass filters.

Standard glasses containing didymium.

Standard glasses containing holmium.

Standard holmium oxide solution.

# Control of absorbance:

In the visible by convention measurements are often made at the wavelength of the 546-nm mercury line.

The Neutral density glasses for 400–1,000 nm.

Copper sulphate in  $\text{H}_2\text{SO}_4$  (0.1 mol/l).

Cobalt ammonium sulphate in  $\text{H}_2\text{SO}_4$  (0.1 mol/l).

Potassium dichromate in  $\text{H}_2\text{SO}_4$  (0.005 mol/l).

Potassium dichromate in perchloric acid (0.001 mol/l).

# British pharmacopoeia 2007:

- Check the absorbance using suitable filters or a solution of *potassium dichromate R* at the wavelengths indicated in Table 2.2.25.-2

Table 2.2.25.-2

| Wavelength<br>(nm) | Specific absorbance<br>$A_{1\text{ cm}}^1$ per cent | Maximum<br>tolerance |
|--------------------|-----------------------------------------------------|----------------------|
| 235                | 124.5                                               | 122.9 to 126.2       |
| 257                | 144.5                                               | 142.8 to 146.2       |
| 313                | 48.6                                                | 47.0 to 50.3         |
| 350                | 107.3                                               | 105.6 to 109.0       |
| 430                | 15.9                                                | 15.7 to 16.1         |

- The tolerance for the absorbance is  $\pm 0.01$ .



# United States pharmacopoeia:

USP

USP

A number of standard inorganic glass filters as well as standard solutions of known transmittances such as potassium dichromate are available.

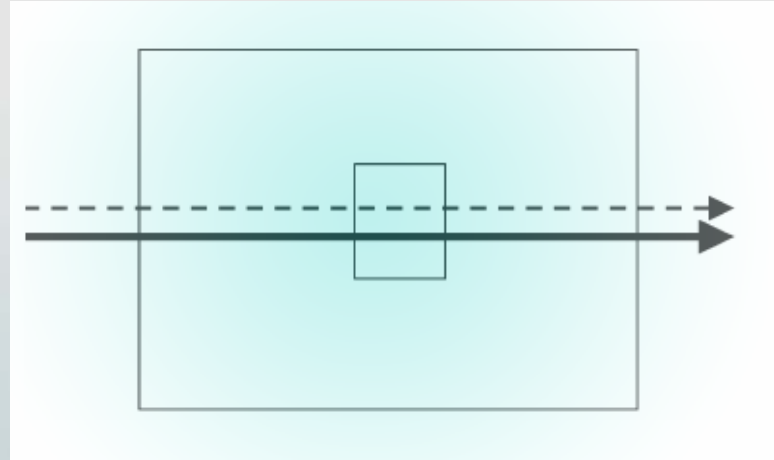
USP

USP

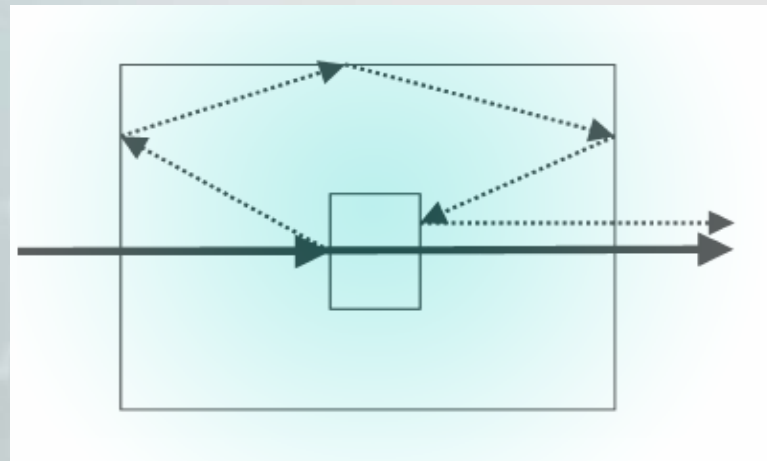
USP

# Limit of stray light:

Heterochromatic stray light



Isochromatic stray light



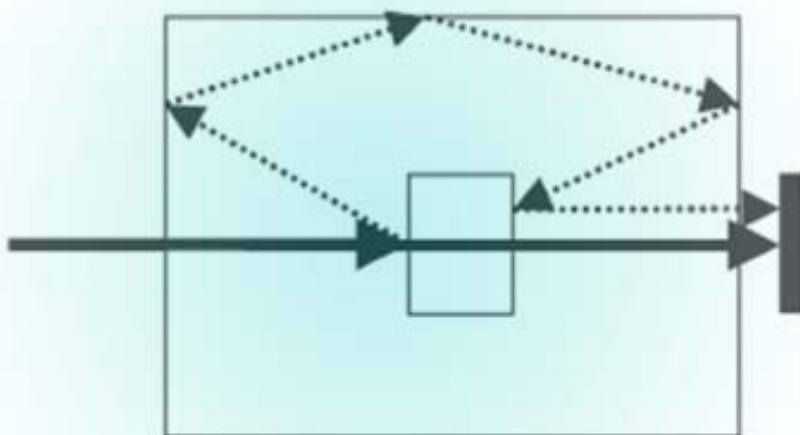
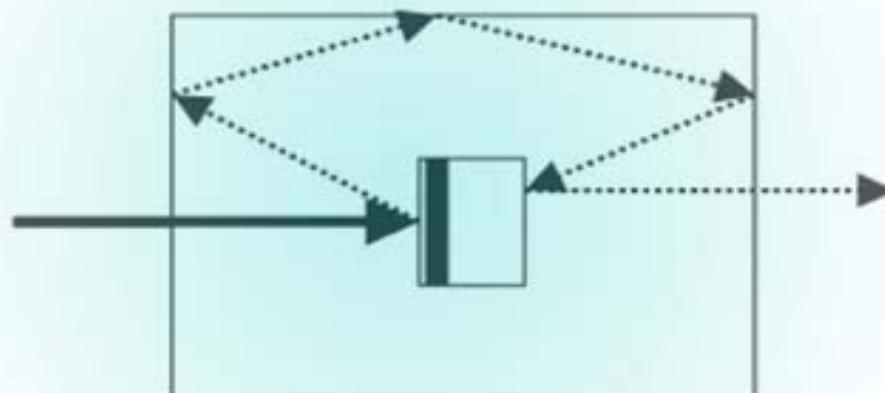
# Heterochromatic stray light:

## 1. Liquid filters:

- KCl aqueous (12 g/l) at 175–200 nm
- NaI aqueous (10 g/l) at 210–259 nm
- Acetone at 250–320 nm
- NaNO<sub>2</sub> aqueous (50 g/l) at 300–385 nm

## 2. Sharp-cut filter glasses.

# Isochromatic stray light:



## British pharmacopoeia 2007:

Stray light may be detected at a given wavelength with suitable filters or solutions: for example the absorbance of a 12 g/l solution of *potassium chloride* *R* in a 1 cm cell increases steeply between 220 nm and 200 nm and is greater than 2.0 at 198 nm when compared with water as compensation liquid. Suitable certified reference materials may also be used.

# Control of cuvettes:

measure the transmittance of water-filled cuvettes against air

- For glass cells at 356 and 650 nm
- For quartz cells at 220 and 240 nm

0.85 < at the lower wavelength

0.88 < at the longer wavelength

## **British pharmacopoeia 2007:**

The tolerance on the path length of the cells used is  $\pm 0.005\text{cm}$ .

When filled with the same solvent, the cells intended to contain the solution to be examined and the compensation liquid must have the same transmittance.

# Wavelength resolution:

The operational qualification of the instrument should then include a test in which the spectra absorbance of a solution of toluene in hexane ( $0.2 \text{ mol l}^{-1}$ ) is measured and the ratio of the absorbance at the 266-nm maximum to that at the 269-nm minimum is determined. This peak-to-valley ratio should be greater than 1.5.

## British pharmacopoeia 2007:

Record the spectrum of a 0.02 percent V/V solution of *toluene R* in *hexane R*. The minimum ratio of the absorbance at the maximum at 269 nm to that at the minimum at 266 nm is stated in the monograph.

Suitable certified reference materials may also be used.

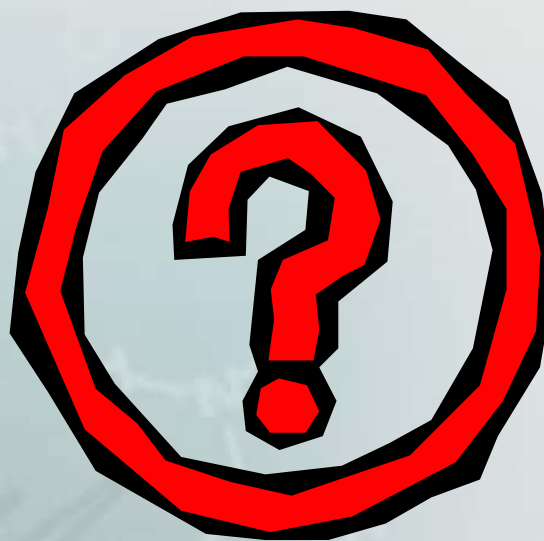
# Spectral slit-width (for quantitative analysis):

To avoid errors due to spectral slit-width, when using an instrument on which the slit-width is variable at the selected wavelength, the slit-width must be small compared with the half-width of the absorption band but it must be as large as possible to obtain a high value of  $I_0$ . Therefore, a slit-width is chosen such that further reduction does not result in a change in absorbance reading.

# References:

- British Pharmacopoeia 2007
- US Pharmacopoeia 30
- Calibration of UV–vis spectrophotometers for chemical analysis /Accred Qual Assur (2005) /John F. Clare
- UV Spectroscopy Techniques, instrumentation,data handling./B.J.Clark, T.Frost and M.A.Russell
- Standards in absorption spectrometry/C.Bugess and A.Knowles

# Questions



A photograph of a misty forest scene. Large, mature trees with dense green foliage line a path that recedes into the distance. The ground is covered in green grass. The atmosphere is hazy and serene, with soft light filtering through the trees.

Thank you  
for your attention

*Early Morning Mist, Central Indiana*