Spectrophotometry Standards

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Abstract

A spectrophotometer, as an analytical tool is used in almost every type of chemical, biological or life science laboratory. The instrument may range in complexity from a simple single beam instrument, right through to dual beam or complex and sometimes highly automated instruments. Some such instruments may also be part of, or built into a system for in-line or process measurement. Irrespective of complexity, all spectrophotometric instruments are based on the fundamentals of the Beer-Lambert law. Like all instrumentation they require regular checking and validation to a greater or lesser extent. The parameters tested for spectrophotometers are photometric accuracy (absorbance linearity), wavelength accuracy, bandwidth and stray light. These checks and validation protocols, ensure confidence in all operational and performance matters and are also mandated in many cases by accreditation and regulatory bodies. These functions are determined by the use of a range of chemical standards. Such standards are formulated to give specific responses, depending on the measurement function being tested. Therefore, it is an imperative that high quality standards be available for performing linearity, wavelength, bandwidth and stray light tests. Such standards should be produced from high quality chemicals, that are fully characterised and should follow the metrological principles that all standards are subject to, including traceability, uncertainty of measurement, accuracy, specification, stability, precision and safety. They must also be available to the user at a price that is fit for purpose. There are a number of high-quality producers of such standards. However, the standards produced by Reagecon are most familiar to the authors, so the description of spectrophotometry standards in this paper relate to those available from Reagecon. We believe that the standards described are cutting edge, complete and form the basis of a template to guide the analyst on what to take into account, during the selection process of fit for purpose standards. The narrative and description of what to look for in spectrophotometry standards is preceded by a brief introduction into the science and technology behind spectrophotometry in very simple detail.

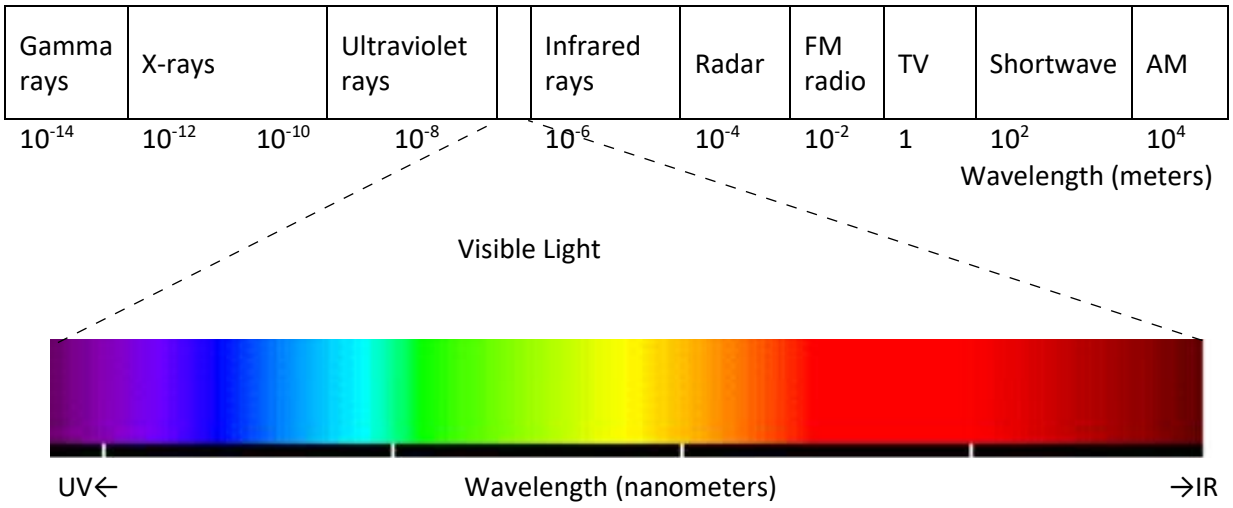
1.0 Introduction

Every chemical compound absorbs, transmits, or reflects light (electromagnetic radiation) over a specific range of wavelengths. Spectrophotometry is a method to measure how much a chemical substance absorbs or transmits light by passing a beam of light through a solution of the substance of interest, and measuring the light intensity emitted. It is a quantitative measurement; the

concentration of an analyte in a solution can be determined by use of the absorbance or transmission properties of a material as a function of wavelength.

Spectrophotometers can measure either visible (white) light or ultraviolet light, down to about 190nm wavelength. Historically, the science of spectrophotometry originated through the study of visible light dispersed according to its wavelength by a glass prism. Graphic 1 shows a schematic of the electro-magnetic spectrum and illustrates the context of both visible light and UV rays within the spectrum compared to other types of radiation.[(1)]

Electromagnetic Spectrum

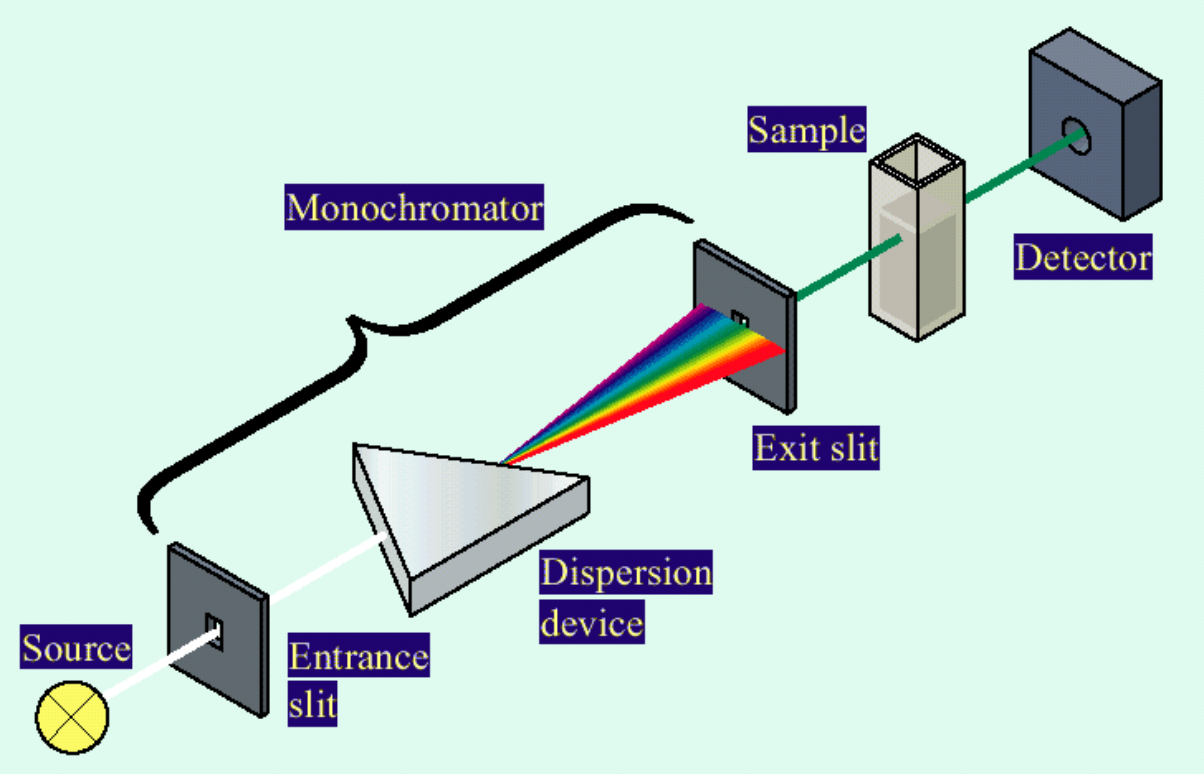


Graphic 1

2.0 Basic Theory on Spectrophotometry

In its simplest form, the main parts of a spectrophotometer are: the light source, a monochromator, a sample chamber (containing the sample of interest) and a detector for analysis. The light from the source passes through an entrance slit in the monochromator which narrows the beam to a useable size. It then passes through a diffraction grating where it is split into bands of monochromatic light. The light then passes through an exit slit which allows light of the selected wavelength to pass through to the sample where some of it is absorbed. Any light passing through the sample is received by the detector. The light energy is converted to electrical energy (voltage) and the resulting voltage fluctuations are analyzed by software, interpreted and presented into an output such as a scan or graph. A schematic of a conventional single beam spectrophotometer can be seen in Graphic 2.

Simplified Graphic Representation of Single Beam Spectrophotometer[2]



Graphic 2

In order for the analyst to have confidence in the performance and measurement accuracy of a spectrophotometer, they must be confident that the following key operational and performance functions are working optimally: Linearity, Wavelength, Bandwidth and Stray Light.[3][4]

The optimization of these four parameters are also mandated by regulatory bodies that include, but are not limited to, USP, Ph. Eur, and ASTM.

These operational and performance functions are determined by use of a series of chemical standards. The standards are formulated from chemicals whose characteristics are proven to give specific responses at particular wavelengths. Spectrophotometer standards are prepared gravimetrically on a weight/weight basis, whereby both solute and solvent are weighed on a calibrated balance.

3.0 Applications of Spectrophotometry Standards

3.1 Linearity

The amount of light absorbed by a sample at any particular wavelength is directly related to the concentration of a sample. This relationship between absorbance and concentration is defined by the

Beer-Lambert Law. The Beer-Lambert Law states that there is a linear relationship between the absorbance and the concentration of a sample.

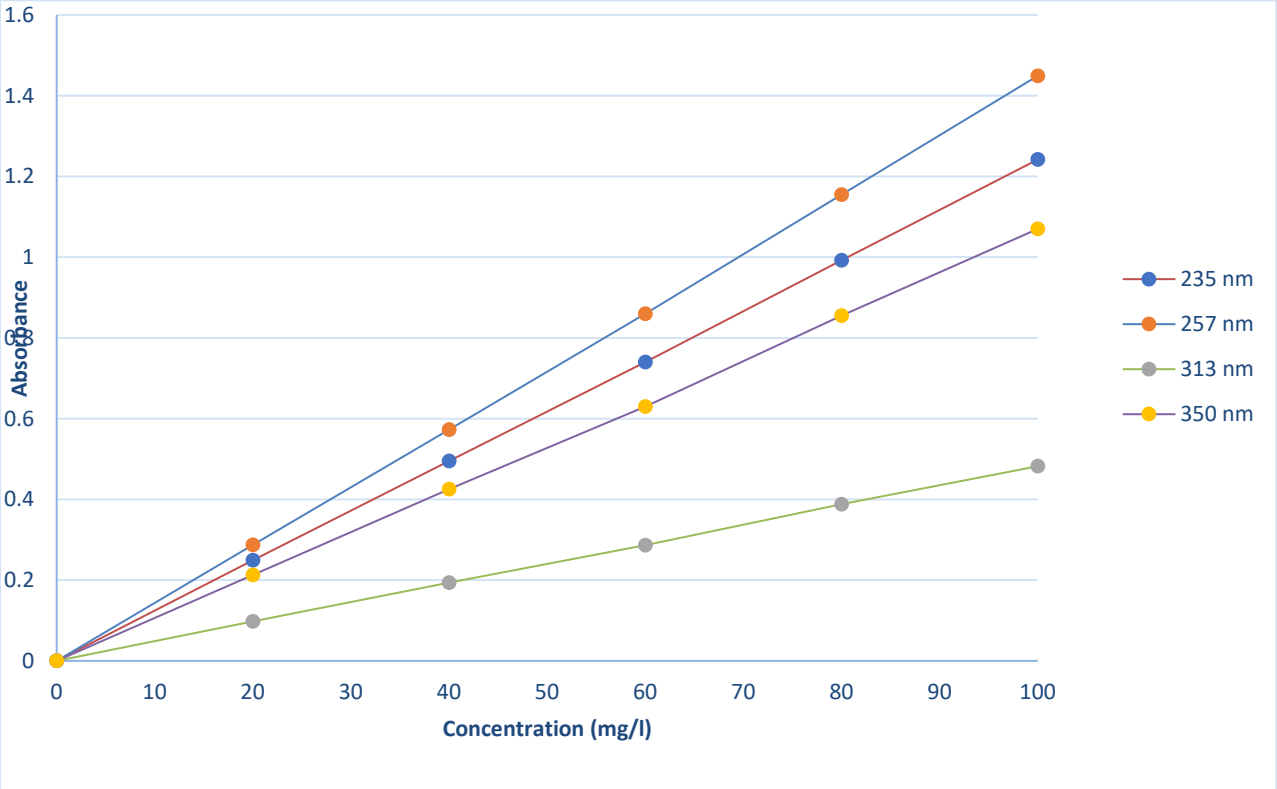
The Beer-Lambert Law for Absorbance (A) can be extended to transmission and expressed as follows:

A = e x b x c

where: e= molar absorptivity, b= pathlength, c= concentration of solution.

Following the Beer-Lambert Law, solutions were identified such as acidic Potassium Dichromate which when diluted in a range e.g. 0-100 mg/l, give a spectral scan at specified wavelengths where the absorbance is a linear function of the standard concentration of Potassium Dichromate (as shown in Graphic 3). Therefore, Potassium Dichromate and other such solutions are used as Linearity Standards.

Absorbance Profile of Potassium Dichromate at concentrations of 0-100nm



Graphic 3

An example of another solution that is regularly used to check linearity is nicotinic acid at a concentration range of 0 – 24 mg/litre at 213 and 261nm wavelength. Although there are a number of highly reputable producers of spectrophotometry standards that offer excellent products in the marketplace, these authors are most familiar with those offered by Reagecon, having been the lead

scientists involved in the development of these standards. Therefore, Reagecon products are referenced throughout this paper, in order to demonstrate, the types of products to be found in the marketplace and to enable the analyst to make the best possible selections, from a procurement perspective. Examples from Reagecon's range of linearity standards, including those that contain Potassium Dichromate and Nicotinic Acid, are presented in Tables 1 and 2. Information is also presented pertaining to pack sizes and packaging format.

Potassium Dichromate Linearity Standards @ 235, 257, 313 & 350nm Wavelength

Product No.	Description	Concentration	Pack Size
RSPEC1022	Potassium Dichromate Linearity Set With Blank in Sealed Cuvettes	0mg/l, 20mg/l, 40mg/l, 60mg/l, 80mg/l, 100mg/l	6 x Permanently sealed UV Cuvettes
RSPEC0022	Potassium Dichromate Absorbance/Transmission Standard	20mg/l	2 x Permanently Sealed UV Cuvettes (including blank)
RSPEC0023	Potassium Dichromate Absorbance/Transmission Standard	40mg/l	2 x Permanently Sealed UV Cuvettes (including blank)
RSPEC0024	Potassium Dichromate Absorbance/Transmission Standard	60mg/l	2 x Permanently Sealed UV Cuvettes (including blank)
RSPEC0025	Potassium Dichromate Absorbance/Transmission Standard	80mg/l	2 x Permanently Sealed UV Cuvettes (including blank)
RSPEC0026	Potassium Dichromate Absorbance/Transmission Standard	100mg/l	2 x Permanently Sealed UV Cuvettes (including blank)
RSPEC00511	Blank - 0.001M Perchloric Acid	0mg/l	100ml Amber Bottle
RSPEC00221	Potassium Dichromate Absorbance/Transmission Standard	20mg/l	100ml Amber Bottle
RSPEC00231	Potassium Dichromate Absorbance/Transmission Standard	40mg/l	100ml Amber Bottle
RSPEC00241	Potassium Dichromate Absorbance/Transmission Standard	60mg/l	100ml Amber Bottle
RSPEC00251	Potassium Dichromate Absorbance/Transmission Standard	80mg/l	100ml Amber Bottle
RSPEC00261	Potassium Dichromate Absorbance/Transmission Standard	100mg/l	100ml Amber Bottle

Table 1 (1)(5)

Nicotinic Acid Linearity Standards @ 213 & 261 Wavelength

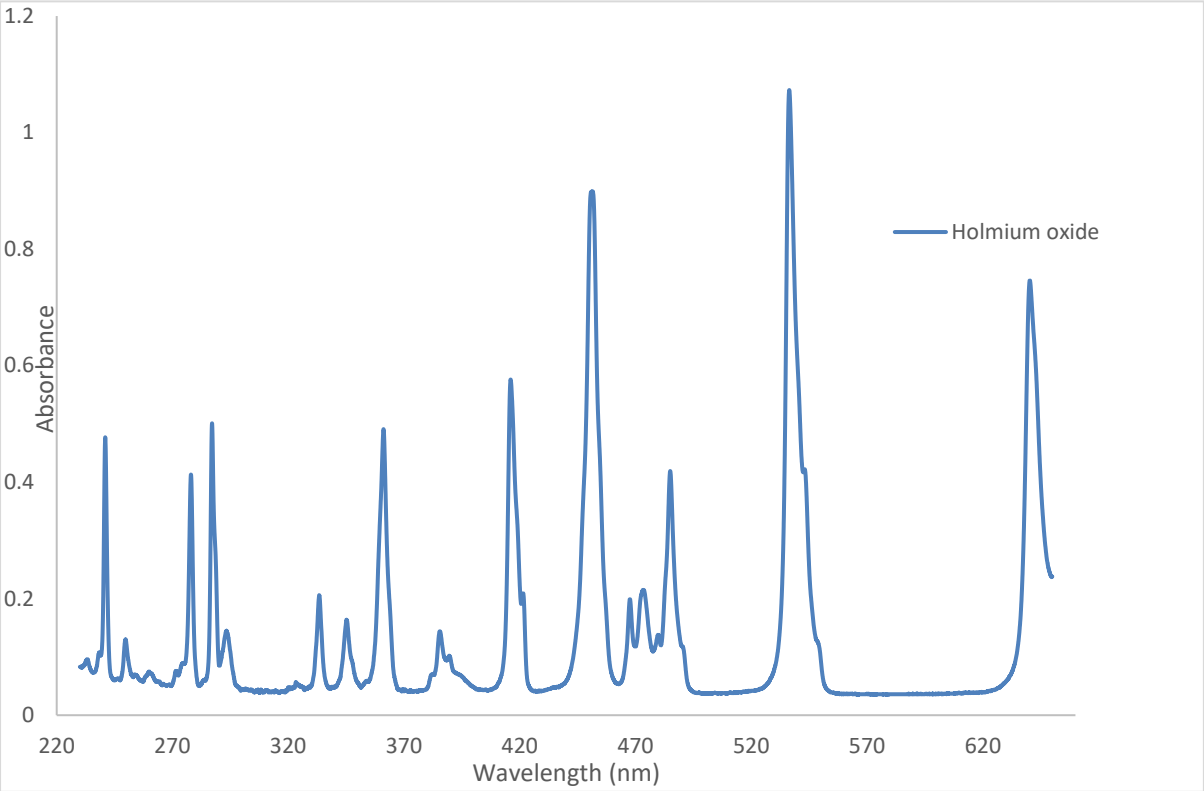
Product No.	Description	Concentration	Pack Size
RSPEC1027	Nicotinic Acid Linearity Set With Blank in Sealed Cuvettes	0mg/l, 6mg/l, 12mg/l, 18mg/l, 24mg/l	5 x Permanently sealed UV Cuvettes (including blank)
RSPEC0027	Nicotinic Acid Absorbance/Transmission Standard	6mg/l	2 x Permanently Sealed UV Cuvettes (including blank)
RSPEC0028	Nicotinic Acid Absorbance/Transmission Standard	12mg/l	2x Permanently Sealed UV Cuvettes (including blank)
RSPEC0029	Nicotinic Acid Absorbance/Transmission Standard	18mg/l	2 x Permanently Sealed UV Cuvettes (including blank)
RSPEC0030	Nicotinic Acid Absorbance/Transmission Standard	24mg/l	2 x Permanently Sealeds UV Cuvette (including blank)
RSPEC00521	Blank -- 0.1M Hydrochloric Acid	0mg/l	100ml Amber Bottle
RSPEC00271	Nicotinic Acid Absorbance/Transmission Standard	6mg/l	100ml Amber Bottle
RSPEC00281	Nicotinic Acid Absorbance/Transmission Standard	12mg/l	100ml Amber Bottle
RSPEC00291	Nicotinic Acid Absorbance/Transmission Standard	18mg/l	100ml Amber Bottle
RSPEC00301	Nicotinic Acid Absorbance/Transmission Standard	24mg/l	100ml Amber Bottle

Table 2 (1)(5

3.2 Wavelength

It is important to be sure that the energy exiting from the monochromator contains light at the wavelength that the test sample absorbs at. This can be checked using wavelength standards. Wavelength standards are chemical mixtures known to give a spectral scan containing a series of characteristic peaks correlating with particular wavelengths of the measuring instrument. For example, when Holmium Oxide is diluted in Perchloric acid, it provides a set of known responses, or peaks at a different specified wavelength. The Standards are used for validation of the wavelength scale of a spectrophotometer in both the UV and visible regions.

Characteristic absorbance plot for Holmium Oxide solution between 230nm and 650nm.



Graphic 4

In addition to the use of Holmium Oxide, the elements Didymium and Samarium may also be used as wavelength standards as can be seen in Table 3.

Reagecon

Wavelength standards from Reagecon.

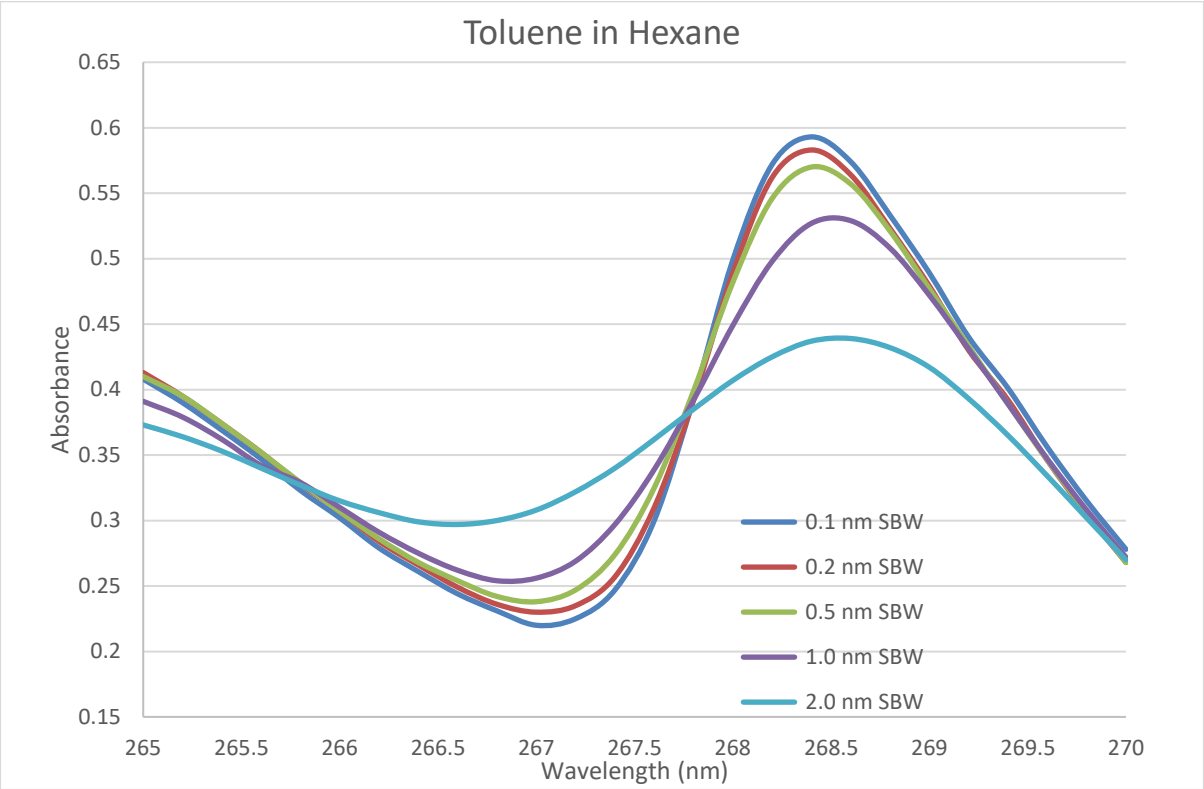
Product No.	Description	Nominal Peak Wavelengths (0.2nm Slit Width)	Pack Size
RSPEC0001	Didymium Solution UV and Visible Wavelength Standard 298nm to 865nm	298nm, 328.8nm, 353.8nm, 443.8nm, 468.5nm, 481.3nm, 511.5nm, 521.6nm, 574.8nm, 731.4nm, 739.6nm, 794nm, 801.1nm, 865nm	1 x Permanently Sealed UV Cuvette
RSPEC0008	Samarium Solution UV and Visible Wavelength Standard 235nm to 480nm	235nm, 278.8nm, 290.1nm, 305.2nm, 317.4nm, 331.6nm, 344.4nm, 362.2nm, 374.1nm, 390.4nm, 401.1nm, 415.3nm, 463.4nm, 478.6nm	1 x Permanently Sealed UV Cuvette
RSPEC0015	Holmium Oxide Solution UV and Visible Wavelength Standard 240nm to 640nm	240.8nm, 249.6nm, 278nm, 286.8nm, 333nm, 345.4nm, 361.1nm, 385.2nm, 416nm, 451.8nm, 467.6nm, 485nm, 536.3nm, 640.2nm	1 x Permanently Sealed UV Cuvette
RSPEC00011	Didymium Solution UV and Visible Wavelength Standard 298nm to 865nm	298nm, 328.8nm, 353.8nm, 443.8nm, 468.5nm, 481.3nm, 511.5nm, 521.6nm, 574.8nm, 731.4nm, 739.6nm, 794nm, 801.1nm, 865nm	100ml Amber Bottle
RSPEC00081	Samarium Solution UV and Visible Wavelength Standard 235nm to 480nm	235nm, 278.8nm, 290.1nm, 305.2nm, 317.4nm, 331.6nm, 344.4nm, 362.2nm, 374.1nm, 390.4nm, 401.1nm, 415.3nm, 463.4nm, 478.6nm	100ml Amber Bottle
RSPEC00151	Holmium Oxide Solution UV and Visible Wavelength Standard 240nm to 640nm	240.8nm, 249.6nm, 278nm, 286.8nm, 333nm, 345.4nm, 361.1nm, 385.2nm, 416nm, 451.8nm, 467.6nm, 485nm, 536.3nm, 640.2nm	100ml Amber Bottle

Table 3 (1)(5)

3.3 Bandwidth

Resolution is the ability of a spectrophotometer to distinguish between two absorbance bands which are close together. This is usually described in terms of the Spectral Bandwidth (SBW) of the instrument, and is related to the range of wavelengths coming from the exit slit of the monochromator. Bandwidth Standards utilize the properties of specific chemicals, such as Toluene in Hexane, that produce a series of characteristic peaks and troughs over a very narrow change of wavelength . The ratio of the absorbance for a solution of Toluene in Hexane at the maximum peak at approx. 269 nm and the minimum trough at approx. 266 nm is determined and compared against specification. If the test fails it is indicative of an issue with the monochromator or detector.

Absorbance plot of Toluene in Hexane from 265nm to 270nm ***at varying Slit Bandwidth (SBW)***



Graphic 5

The products, part numbers and package presentations available from Reagecon for Bandwidth verification are presented in Table 4.

Reagecon Bandwidth Standards

Product No.	Description	Certified Value	Packed in
RSPEC1031	Toluene in Hexane Bandwidth Standard	Ratio of 268.7nm peak to 266.8nm trough	2 x Permanently sealed UV Cuvettes (including blank)
RSPEC00311	Bandwidth Standard - Toluene in Hexane	Ratio of 268.7nm peak to 266.8nm trough	100ml Amber Bottle
RSPEC00531	Bandwidth Standard - Blank	Ratio of 268.7nm peak to 266.8nm trough	100ml Amber Bottle

(1)(5

Table 4

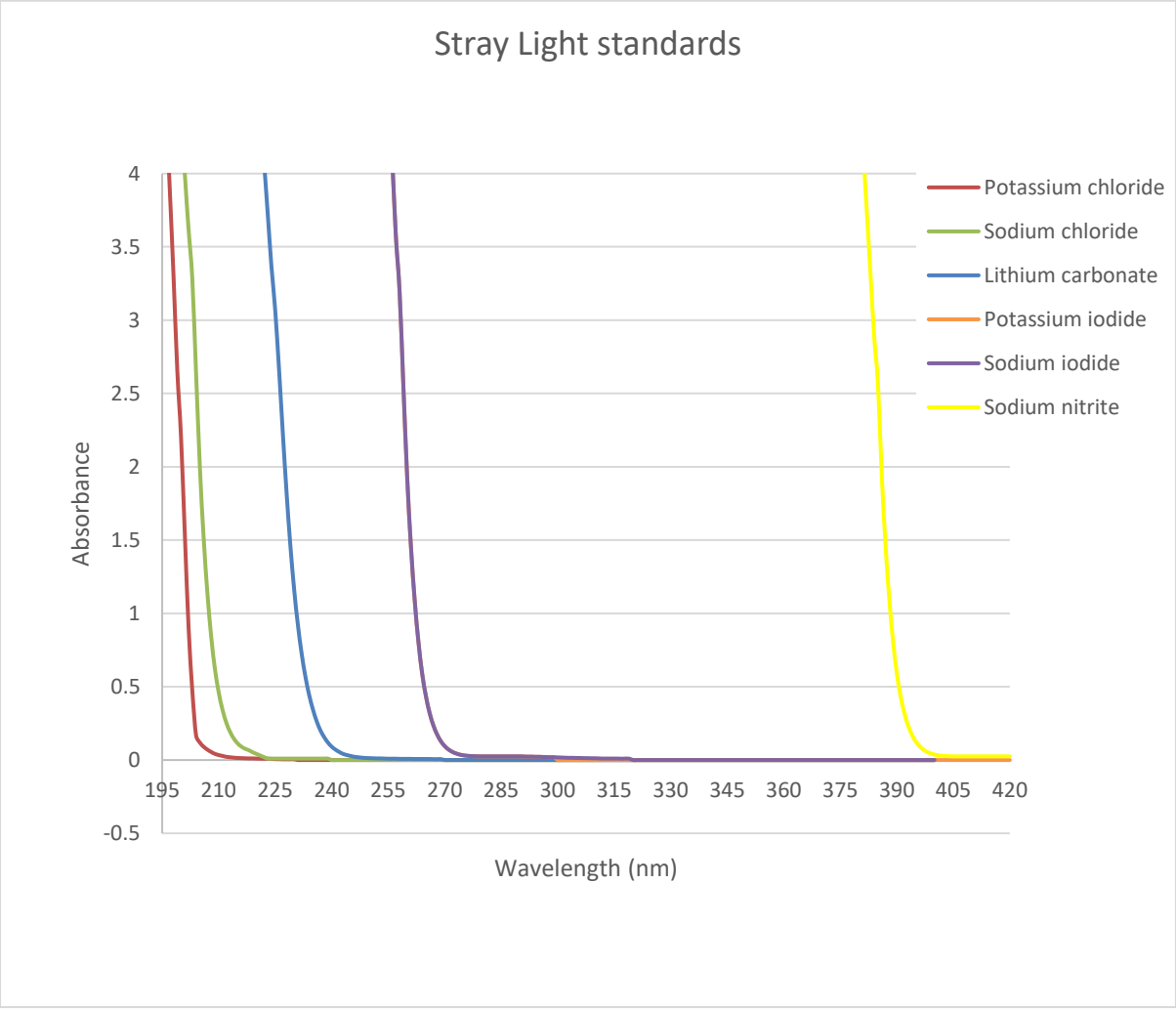
3.4 Stray Light

Stray light is defined as any light that is detected by the spectrophotometer which does not belong to the given bandwidth of the sample being measured. Often the result of light scattering, diffraction or errors with the instrument, stray light can cause a variety of distortions and errors in analysis. In order to measure stray light, standards are needed that absorb all light of the wavelength at which the measurement is to be performed and then cut off at a specified wavelength.

Stray light Standards consist of alkali metal halide solutions which are known to give a zero-absorbance reading at a specified wavelength (cut off point). Graphic 6 below shows the cut-off wavelength for a variety of stray light standards. At the cut off wavelengths any absorbance reading seen indicates that the instrument is measuring light from a source other than that which passed through the sample cuvette.

Other chemicals used for this application include Sodium Chloride, Lithium Chloride, Sodium Nitrate and Sodium Iodide.

Absorbance plot of Stray Light standards at varying wavelengths



Graphic 6

The Reagecon range of stray light standards is presented below in Table 5.

Reagecon Stray Light Standards at different wavelengths

Product No.	Description	Cut Off	Packed in
RSPEC0036	Stray Light Inorganic Cut-off filter - Sodium Nitrite	390nm	2 x Permanently sealed UV Cuvettes (including blank)
RSPEC0037	Stray Light Inorganic Cut-off filter - Potassium Iodide	260nm	2 x Permanently Sealed UV Cuvettes (including blank)
RSPEC0038	Stray Light Inorganic Cut-off filter - Sodium Iodide	260nm	2 x Permanently Sealed UV Cuvettes (including blank)
RSPEC0039	Stray Light Inorganic Cut-off filter - Lithium Carbonate	227nm	2 x Permanently Sealed UV Cuvettes (including blank)
RSPEC0040	Stray Light Inorganic Cut-off filter - Sodium Chloride	205nm	2 x Permanently Sealed UV Cuvettes (including blank)
RSPEC0041	Stray Light Inorganic Cut-off filter - Potassium chloride	200nm	2 x Permanently Sealed UV Cuvettes (including blank)
RSPEC00541	Stray Light Blank - Aqueous		100ml Amber Bottle
RSPEC00361	Stray Light Inorganic Cut-off filter - Sodium Nitrite	390nm	100ml Amber Bottle
RSPEC00371	Stray Light Inorganic Cut-off filter - Potassium Iodide	260nm	100ml Amber Bottle
RSPEC00381	Stray Light Inorganic Cut-off filter - Sodium Iodide	260nm	100ml Amber Bottle
RSPEC00391	Stray Light Inorganic Cut-off filter - Lithium Carbonate	227nm	100ml Amber Bottle
RSPEC00401	Stray Light Inorganic Cut-off filter - Sodium Chloride	205nm	100ml Amber Bottle
RSPEC00411	Stray Light Inorganic Cut-off filter - Potassium chloride	200nm	100ml Amber Bottle

(1)(5)

Table 5

3.5 Pharmacopoeia requirements

All of the various pharmacopoeia requires users to demonstrate that their spectrophotometry instrumentation is working correctly with respect to the aforementioned operational parameters of Linearity, Wavelength, Bandwidth and Stray Light. Although there has been some harmonization of test methods between the various pharmacopeia over the years, there are subtle differences between them in terms of how compliance with the key operational parameters is currently demonstrated. For example, USP 857 outlines the use of potassium dichromate solutions for checking the control of absorbance. By comparison, the Ph. Eur. mandates the use of nicotinic acid solutions for this task. There are also slight differences in the methodology outlined in each of the pharmacopoeia for testing stray light, with the USP specifying measurement be performed using a 5 mm cuvette for the reference and a 10 mm cuvette for the test solution. Despite these differences, the spectrophotometric solutions produced by Reagecon can be used to demonstrate compliance with the various pharmacopoeia requirements.

Details of the various pharmacopoeia standards are presented in Table 6.

Reagecon Pharmacopoeia UV standards

Bandwidth Standards (Ph. Eur.)	
Product No.	Description
RSPEC-EP00631	Reagecon Spectrophotometry Bandwidth Standard Toluene in Hexane (Ph. Eur) Ratio of 268.7nm Peak to 266.8nm Trough
RSPEC-EP00731	Reagecon Spectrophotometry Bandwidth Standard Blank (Ph.Eur) Ratio of 268.7nm Peak to 266.8nm Trough

Linearity Standards (Ph. Eur.)	
Product No.	Description
RSPEC-EP00651	Reagecon Spectrophotometry Nicotinic Acid Absorbance/Transmission (Linearity) Standard 12 mg/L (Ph.Eur)
RSPEC-EP00661	Reagecon Spectrophotometry Nicotinic Acid Absorbance/Transmission (Linearity) Standard 6 mg/L (Ph.Eur)
RSPEC-EP00671	Reagecon Spectrophotometry Nicotinic Acid Absorbance/Transmission (Linearity) Standard 18 mg/L (Ph.Eur)
RSPEC-EP00681	Reagecon Spectrophotometry Nicotinic Acid Absorbance/Transmission (Linearity) Standard 24 mg/L (Ph.Eur)
RSPEC-EP00751	Reagecon Spectrophotometry Absorbance/Transmission (Linearity) Standard Blank - 0.005M Sulphuric Acid (Ph.Eur)

Wavelength Standards (Ph. Eur.)	
Product No.	Description
RSPEC-EP0064	Reagecon Spectrophotometry Holmium Oxide UV and Visible Wavelength Standard 240nm to 640nm (Ph. Eur)
RSPEC-EP00641	Reagecon Spectrophotometry Holmium Oxide UV and Visible Wavelength Standard 240nm to 640nm (Ph. Eur)

4.0 Conclusion

A spectrophotometer is a highly sophisticated piece of laboratory instrumentation, that has application in a wide range of scientific disciplines. Such an instrument is to be found in almost every laboratory worldwide. Like almost all scientific instruments, calibration, quality control, method validation and qualification, are mandatory, either from a good laboratory practice perspective or regulatory requirement. To perform any, or all, of these metrological functions, the use of a high quality standard is an imperative.

It is hoped that this paper, outlines in simple terms, the principles of how a spectrophotometer works, and the appropriate checks that must be performed. It is also hoped that the ranges of standards

offered are comprehensive, and enable the analyst to obtain the correct analytical result and prove the correctness of that result. The features and benefits of a good spectrophotometry range of standards should include the features presented in Graphic 7.

Features and Benefits of High-Quality Spectrophotometry Standards
Can be used with any UV/VIS Spectrophotometer brand
NIST Traceable
Supplied as permanently sealed cuvettes or ready to use solutions in 100ml bottles
Sealed cuvettes can be recertified offering an indefinite operational lifespan
Certified at multiple wavelengths (in accordance with function performed whether that be Linearity, Wavelength, Bandwidth or Stray Light)
Measurement uncertainties reported on every certificate of analysis

Graphic 7

The producer of high-quality standards should hold ISO 17025 accreditation for calibration of laboratory balances. The resulting Balance Certificate of Calibration should be issued in accordance with the requirements of ISO/IEC 17025. The certified values of each standard should then be verified using a high-performance spectrophotometer calibrated with NIST traceable, ISO 17034 Certified Reference Standards, where possible.

5.0 Bibliography

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