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Sample Analysis Report

Number 70

Biopharmaceutical Quality Control Applications of the Cary 50

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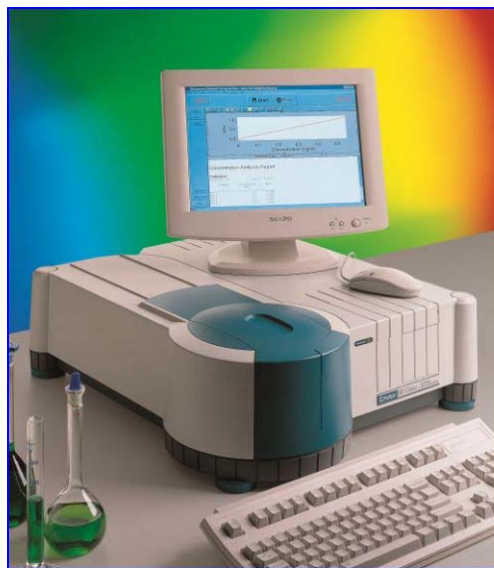
Introduction

Pharmaceuticals manufacturing often includes stringent quality controls standards including standards for the UV optical properties of the product. In some cases the UV/Visible spectroscopy is confirmation of the purity and concentration of the product. This experiment was to demonstrate that the Cary 50 was a suitable platform for determining these parameters for applications within a regulated biopharmaceuticals company.

Instrumentation

The Very small beam geometry, 1.5 nm bandwidth, and very high intensity flash source of the Cary 50, coupled with a long pathlength Czerny-Turner monochromator design and ultra-fast scanning capability, make the Cary 50 a uniquely powerful instrument when coupled to fiberoptics devices. The ease of use is further enhanced due to the room-light immunity of the system, which removes the need to close the sample compartment when taking a reading. This enables the Cary 50 to read samples, which would otherwise be too large for sample compartment mounting. The standard Cary 50 offers capabilities for reading microvolumes in a specially designed 1-5 μ L cuvette as well as providing high precision in standard cuvettes. Amongst a wide range of accessories available for the Cary 50 are fiberoptics probes, which enable readings to be made remote from the instrument and an 18 position, temperature controlled autochanger, which is able to automate analysis of single-point or multiple readings (kinetics) samples.

The Cary 50 uses a Xenon Flash source lamp, which is so durable it should never need replacement. The instrument requires no warm-up time, and the light beam, although intense during the sub-microsecond flash, is unlikely to photo-degrade sensitive samples as the average intensity is low, and the sample is only ever illuminated with monochromatic light.



A Cary 50 Probe spectrophotometer with 18 position autochanger was used with either quartz glass or plastic disposable cuvettes, 10mm pathlength. The scan software module was used to obtain data for the range 200-700 nm (most of the UV/ visible spectrum) and the Concentration module used to obtain data for a series of standards, calibrate and plot the curve for these standards and to give the concentration of a test sample from the calibration data.

Conditions

The Cary 50 is a dual-beam instrument but only the sample beam is accessible for taking readings. The dual-beam design gives the Cary 50 exceptional stability and room-light immunity, which makes measurements simple and very rapid, compared with conventional designs, which require the sample compartment to be closed between readings. All readings were taken in the Cary 50 in normal operating mode with the sample compartment doors open and the samples being presented in the 18 position autochanger.

Results

Figure 1 shows baseline noise from the scan of a test compound at approx 600nm. Noise error is approximately 0.00025A (+-) with a signal averaging of 0.1 seconds, 1200nm/min scan speed.

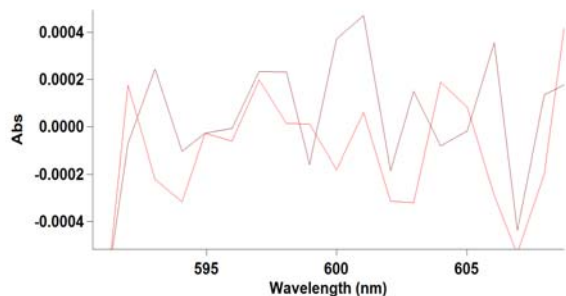
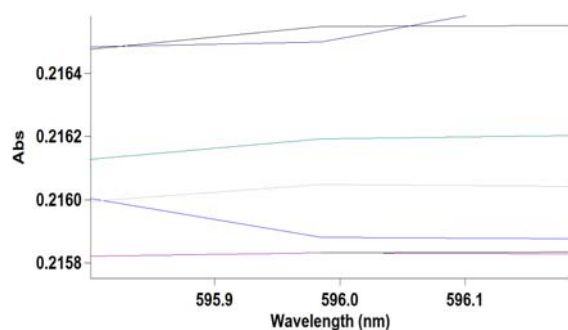


Figure 2 shows detail from the apex of the 596 nm peak absorbance of the compound scanned. 6 scan traces overlaid and noise/reproducibility of traces shown



The repeatability of the readings is 0.2162A +/- 0.0004A or better at 0.2 Abs.

Figure 3 shows the peak shape and overlay of six repeats of the scan over the nominal return to baseline area of this component peak. (6 scans overlaid with 2 baseline scans also shown)

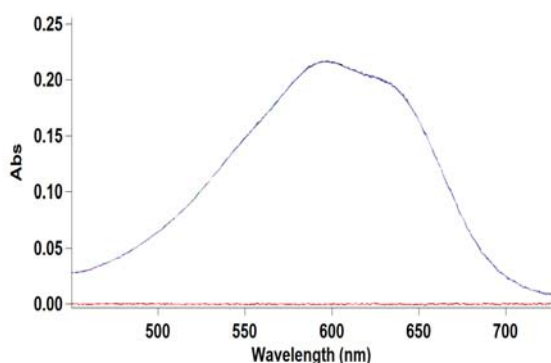
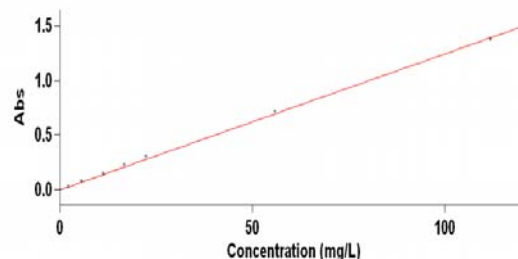


Figure 5 shows the calibration curve produced using a series of six standards, measured twice each.



Concentration analysis report for this experiment,

Concentration Analysis Report

Report time 05/12/2007 16:13:06
 Method C:\Documents and Settings\Owain McKenzie\Desktop\BRB4.MCN
 Batch name C:\Documents and Settings\Owain McKenzie\Desktop\BRB4A4.SCN
 Application Concentration 4.00 (337)
 Operator

Instrument Settings

Instrument Cary 50
 Wavelength (nm) 600.0
 Ordinate Mode Abs
 Ave Time (sec) 0.1000
 Replicates 2
 Standard/Sample averaging OFF
 Weight and volume correction OFF
 Fit type Linear Direct
 Min R² 0.95000
 Concentration units mg/L
 Cell changer ON

Comments:

Zero Report

Read	Abs	nm
Zero	(0.1259)	600.0

Calibration

Collection time 05/12/2007 16:13:20

Standard	Concentration, F mg/L	Mean	SD	%RSD Readings
Std 1	0.0000	-0.0001	0.0001	-93.00001
Std 2	0.0001	-0.0010	0.0000	-1.40010
Std 3	2.2000	0.0211	0.0000	0.0311
Std 4	5.6000	0.0757	0.0002	0.270755
Std 5	11.2000	0.1450	0.0002	0.151488
Std 6	16.8000	0.2245	0.0000	0.002245
Std 7	22.4000	0.3050	0.0001	0.053055
Std 8	55.9000	0.7153	0.0001	0.027152
Std 9	111.9000	1.3742	0.0012	0.0913733

Calibration eqn Abs = 0.01245 * CONC
 Correlation Coefficient 0.99925

Discussion of Results

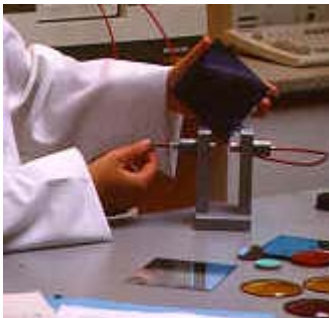
These experiments aim to demonstrate that the Cary 50 is a high precision research grade spectrophotometer, which is able to meet the demands of the Biopharmaceuticals industry for accuracy, precision and repeatability.

The Cary 50 demonstration showed that it is able to perform scans at high speed, which shows variance of less than 0.0005 Abs at peak absorbance and approximately 0.0002 Abs at baseline. Calibration standards provided for analysis in disposable plastic cuvettes showed repeatability of better than 0.0004 Abs at concentrations below 1.6 Abs and still better than 0.002Abs at 1.6 Abs.

The Cary 50 can therefore equal and in most cases exceed the performance of many ostensibly higher specification instruments and yet has many advantages arising from the use of the xenon flash lamp (as described in the introduction.)

21CFR11 software is available in addition to the standard GLP software module. Full documentation and Installation/operator qualification training is also available.

Cary 50 Accessories include 18 position autochanger, fiberoptics in both dip-probe (photo) and transmission probe (illustration) configurations, temperature control, cell stirring, 18 position autochanger and many more.



These data are the actual experimental results obtained on Wednesday 5th December 2007

For further information, contact your local Varian Sales Office. 01865 291500(UK)