GENOVA NANO Application note: A09-012A

Accuracy and precision of the Genova Nano

Introduction

The Jenway Genova Nano spectrophotometer combines a micro volume accessory with the dedicated life science measurement modes of the Genova Plus as well as those of a standard spectrophotometer. The Genova Nano is able to measure sample volumes as low as 0.5µl with a high degree of accuracy, reproducibility and speed. This ability conserves precious samples, reduces the need for dilution and eliminates the requirement for cuvettes. Cleaning is quick and simple: wiping the read heads with a lint-free cloth removes all trace of the sample, allowing faster change over between samples and therefore increasing sample throughput.

Fig. 1: Applying samples to the read head of the micro volume accessory.

An important requirement for measurement of micro volumes is accuracy and precision. Accuracy is defined as how close the measured parameter is to a defined value and precision is a measurement of repeatability or reproducibility. The Genova Nano is specified to an absorbance accuracy of +/-2% at 260nm and a precision of < 0.005A between 0 and 1A (at 0.5mm path length). This application note demonstrates this specification on eight individual units and also shows concentration reproducibility when measuring in dsDNA mode.

Materials and Methods

Each of the Genova Nano units was calibrated using the certified calibration standard solution provided following the instructions detailed in the user manual.

To determine absorbance accuracy, the unit was set up in multiwavelength mode to measure at 260nm and 330nm. The unit was zeroed using 2µl of the calibration blank solution then 2µl aliquots of the calibration standard solution were measured. Following each measurement, the sample was wiped off the read head using a lint-free wipe. This test was performed at both at 0.5mm and 0.2mm path lengths and on each individual unit. 10 successive readings were used for data analysis.

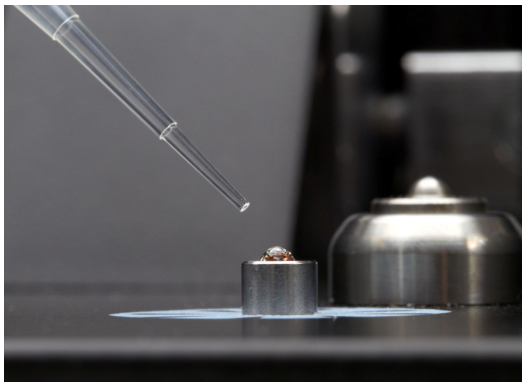

Fig. 2: A droplet of sample is shown on the Genova Nano read head.

In order to measure absorbance precision or repeatability, a sample of green food colouring (Supercook, Leeds, UK) was used. This contains a mix of tartrazine (E102) and Green S (E142). The absorbance spectrum

of the food colouring shows a peak at 430nm and a trough at 520nm (Fig. 3), therefore these wavelengths were chosen for measurement. The food colouring was diluted with water to give an absorbance between 0 and 1 in a path length of 0.5mm.

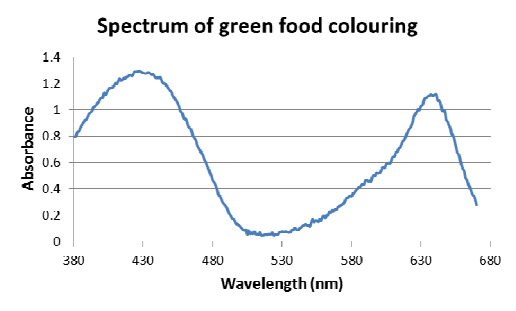

Fig. 3: Visible spectrum of green food colouring measured using the microvolume accessory at 0.5mm path length showing a peak at 430nm and trough at 520nm.

In a similar manner to the absorbance accuracy test, the unit was set up in multiwavelength mode, however this time measurements were made at 430nm and 520nm. The instrument was zeroed using 2µl of water then 2µl aliquots of the food colouring solution were measured. Following each measurement, the sample was wiped off the read head using a lint-free wipe. This test was performed at 0.5mm path length on each individual unit. 10 successive readings were used for data analysis.

For measurement of concentration repeatability, the unit was set up in the Life Science Multiwavelength mode with the following parameters:

a. Wavelength 1 = 260
b. Wavelength 2 = 280
c. Wavelength 3 = 230
d. Wavelength 4 = 330
e. Sum = (xF1*(A1-A4))-(xF2*(A2-A4))
f. F1 = 50 for dsDNA
g. F2 = 0
h. Units = µg/ml

The sample used was Calf thymus DNA (Sigma, D3664) diluted to approximately 100µg/ml with nuclease-free water. Measurements were performed as described previously. The instrument was zeroed using 2µl of water then 2µl aliquots of the DNA solution were measured. Following each measurement, the sampls was wiped off the read head using a lint-free wipe. This test was performed at 0.5mm path length on each individual unit. 10 successive readings were used for data analysis.

Results

In order to minimise any factors which may interfere with a reading it is recommended, when performing micro volume measurements, that a reading is also made at a second reference wavelength (where the absorbance of the sample is very low and unchanging) in order to perform a background correction. In each of the measurements shown here, background correction was performed at 330nm for the calibration solution and DNA and at 520nm for the green food colouring. For analysis the absorbance at the background wavelength was subtracted from that of the measurement wavelength.

Results for the absorbance accuracy tests are shown in Fig. 4.

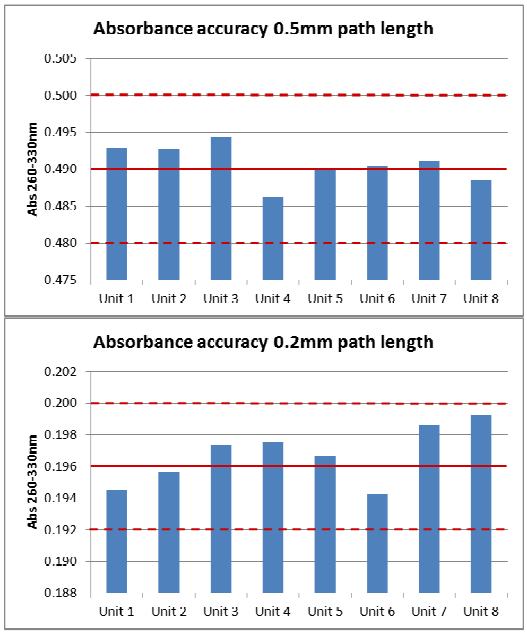

Fig 4: Absorbance accuracy tests on 8 individual Genova Nano units at 0.5mm path length (top) and 0.2mm path length (bottom). The centre unbroken line on each graph represents the expected absorbance value of the standard solution. The broken lines are set +/- 2% from the expected value. The mean of 10 consecutive readings are shown.

The expected absorbance values for the calibration standard solution were 0.490 at 0.5mm path length and 0.196 at 0.2mm path length, according to the certificate supplied with the reagents. All units were well within the specification set at +/-2% of the expected values at both 0.5 and 0.2mm path lengths. In addition there was less than 2% variation between instruments at 0.5mm path length.

To determine repeatability of measurement, a series of food colouring samples were measured and the maximum and minimum reading of the range determined. The results are shown in Fig. 5 and demonstrate highly reproducible results with a range of ≤0.005Abs for 10 consecutive sample readings.

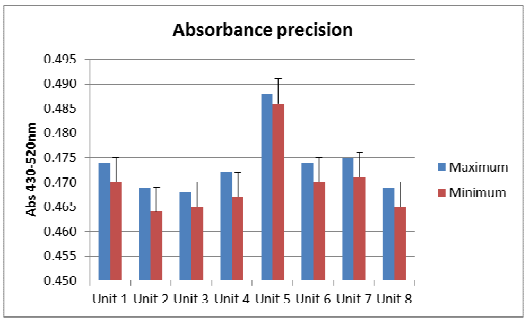

Fig. 5: Absorbance precision tests on 8 individual Genova Nano units. The bars represent the maximum (blue) and minimum (red) values of 10 consecutive readings. The error bar above the minimum values is set to 0.005Abs.

When measuring micro volume samples, very small changes in absorbance can lead to much greater differences in calculated concentration values due to the inherent "dilution" factor of the small path length (20x for the 0.5mm path length and 50x for 0.2mm). Therefore reproducibility in concentration measurements is an important feature. For example, when measuring DNA, an absorbance change of just 0.001 equates to a derived concentration change of 1µg/ml at 0.5mm path length (based on 1 A_{260} unit of dsDNA = 50µg/ml) and 2.5µg/ml at 0.2mm.

A sample of DNA of approximately 100µg/ml was measured using the Life Science multiwavelength mode of the Genova Nano and two representative results are shown in Fig. 6. The results illustrate that a variation of less than +/-2µg/ml was obtained in ten consecutive sample readings. This illustrates further the reproducibility of the Jenway Genova Nano.

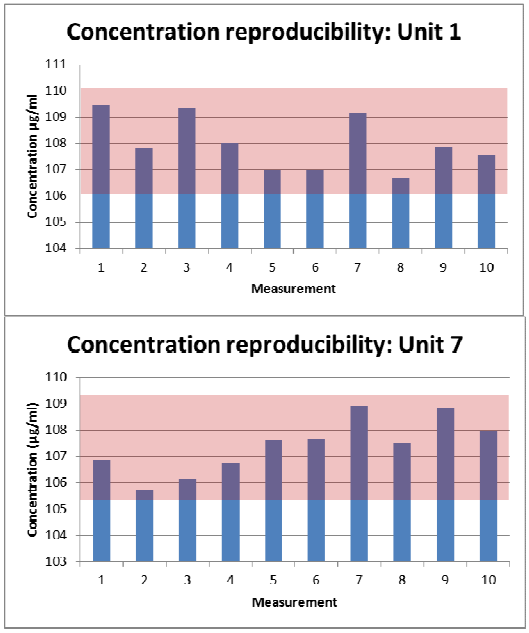

Fig. 6: Concentration reproducibility of the Genova Nano. 10 consecutive readings are shown. The shaded area represents a range spread of +/-2µg/ml, centred over the mid-point of the range.

Conclusions

This application note demonstrates that the Genova Nano is extremely accurate and precise in terms of absorbance measurement both within and between instruments. The ability of the Genova Nano to measure small sample volumes pipetted directly onto the read head, introduces considerable cost savings in terms of time, sample and consumables.

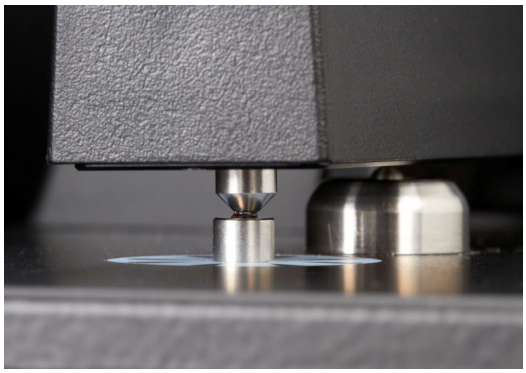

Fig. 7: The Genova Nano read head in reading position.

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