Application Note
Pharmaceuticals

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Benefits of a Unique Multichannel Sipper Flow-Cell Pump for UV-Vis Measurements

Time savings and measurement precision improvements observed when comparing Sipper to manual cuvette measurements

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Introduction

Increasing the throughput of analytical systems that perform routine measurements in a laboratory can save time and money. However, the increase in throughput cannot be at the expense of analytical accuracy or precision. When considering efficiency using UV-Vis spectrophotometers, sample handling is typically a major limitation when manually filling, handling, and cleaning cuvettes.

In this study, the use of a peristaltic pump, designed to pump liquid samples through flow cells, positioned in the spectrophotometer, was compared to manual measurements. The accessory, the Cary Sipper, can be controlled from the spectrophotometer's software, allowing it to be an integrated part of the analytical method.

The Cary Sipper is unique in that it has been designed with three channels, allowing for simultaneous pumping of three liquid samples into the UV-Vis spectrophotometer. This complements the simultaneous measurement capability of the Cary 3500 UV-Vis system and enhances the clear benefits of sample automation.

A common analytical application—the quantitation of vitamin C (L-ascorbic acid) in commercially available effervescent tablets was used for the comparison study.

Experimental

Preparation of standards

A stock solution of L-ascorbic acid was prepared by dissolving 50.0 milligrams of neat L-ascorbic acid in 100 mL of 0.1 M HCl at 23.5 °C to yield a 500 mg/L stock solution at a pH of 1.5. The stock solution was then diluted using 0.1 M HCl to prepare eight standards of concentrations between 0 to 70 mg/L, as shown in Table 1. These standards cover an absorbance range of approximately 0 to 4 absorbance units, which is representative of the range of a typical routine scanning UV-Vis spectrophotometer. The same standard solutions were used for all measurements (with and without the Cary Sipper).

Table 1. Concentrations of prepared standards and their average measured absorbance (n=3).

Standard ID	Concentration (mg/L)	Absorbance
Standard 1	0	0.0014
Standard 2	10	0.5893
Standard 3	20	1.1584
Standard 4	30	1.7088
Standard 5	40	2.2768
Standard 6	50	2.8228
Standard 7	60	3.4147
Standard 8	70	3.9120

Preparation of samples

Commercially available effervescent vitamin C tablets were purchased from a local pharmacy. The label indicated that each tablet contained 1000 milligrams of vitamin C. The weight of each of 20 tablets was recorded (Table 2) and each tablet was then crushed into a powder using a mortar and pestle. Varying amounts, between 5.5 to 28.0 mg, were taken of the resultant powder for each sample. The powder was dissolved in 100 mL of Milli-Q filtered water and set to a pH of 1.5 at 23.5 °C.

This produced 20 samples that were within the calibration range of 0 to 70 mg/L. The same sample solutions were used for all measurements (with and without the Cary Sipper).

Assuming 1000 mg of vitamin C was present in each tablet, as per the product label, the amount of vitamin C in each sample solution was calculated. This calculation was based on the weight of each tablet and the weight of the crushed tablet powder used to make up each sample solution. The final concentration of each sample solution was also calculated. Both calculated values are presented in Table 2.

Table 2. The weight of each of the 20 vitamin C tablets and the weight of powdered tablet used to make up each sample.

Sample No.	Tablet Weight (mg)	Amount Taken (mg)	Calculated Vit C Content in Sample Solution (mg)	Calculated Vit C Concentration in Sample Solution (mg/L)
1.	4209	16.6	3.9	39.4
2.	4253	19.0	4.5	44.7
3.	4239	28.0	6.6	66.1
4.	4212	11.9	2.8	28.3
5.	4247	5.5	1.3	13.0
6.	4239	18.0	4.2	42.5
7.	4238	22.1	5.2	52.1
8.	4231	16.5	3.9	39.0
9.	4242	28.0	6.6	66.0
10.	4201	7.5	1.8	17.9
11.	4219	18.4	4.4	43.6
12.	4229	18.0	4.3	42.6
13.	4214	6.5	1.5	15.4
14.	4219	24.0	5.7	56.9
15.	4261	20.8	4.9	48.8
16.	4209	13.8	3.3	32.8
17.	4234	15.0	3.5	35.4
18.	4241	13.0	3.1	30.7
19.	4268	17.6	4.1	41.2
20.	4229	8.0	1.9	18.9

Instrumentation

An Agilent Cary 3500 Multicell UV-Vis spectrophotometer was used for this study. This configuration of the Cary 3500 instrument allows up to eight cuvette positions to be measured at the same time (seven samples and a reference). For half of the measurements, the instrument was fitted with the Cary Sipper (refer to Figure 1) that can pump three sample solutions simultaneously through three flow cells situated inside the instrument sample compartment. The other half were manually transferred to cuvettes and then to the spectrometer.

Figure 1. The Cary Sipper accessory, connected to the Cary 3500 Multicell UV-Vis spectrophotometer.

Sipper measurements were performed by inserting the Sipper inlet tube into a 15 mL falcon tube containing the solution being analyzed. The solution was then pumped into a single 10 mm pathlength, 390 µL quartz flow cell. Milli-Q water was used for rinsing the flow cell between scans to avoid cross contamination. The measurement of each standard and sample was repeated three times.

The Cary Sipper pump operates at a fixed speed of 80 rpm. The length of time required to pump the solutions through the flow cell prior to measurement is called the 'Fill' time. The subsequent period of no pumping, to allow the solution to settle, is called the 'Hold' time. The final setting of the Sipper is the length of time for the rinse solution to be pumped through the cell. This is called the 'Rinse' time. All three times are set within the Cary UV Workstation software and can be saved as part of a stored method.

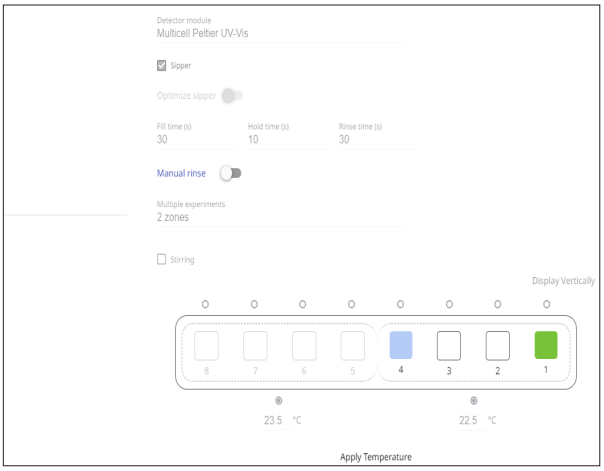

Figure 2. The controls for the Cary Sipper accessory within the Cary UV Workstation software.

For the other half of the measurements, standard 10 mm pathlength, 3.5 mL quartz cuvettes were used. They were manually filled with sample solution and rinsed after each measurement with Milli-Q water. The measurement of each standard and sample was repeated three times.

The same standard and sample solutions were used for both instrument setups, allowing a direct comparison of the results.

Measurements were performed using the Concentration application within the Cary UV Workstation software. This application provides a method allowing for creation of a calibration curve and determination of the concentration of samples, based on the calibration curve.

A wavelength scan of each standard and sample (using the parameters listed in Table 3) was performed from 350 to 200 nm. The samples were baseline-corrected using 0.1 M HCl. The peak at 243 nm was used for the quantitation. The resultant absorbance values for each standard were then used to create the calibration curve. The samples were measured using the same instrument parameters and vitamin C content of each sample was quantified.

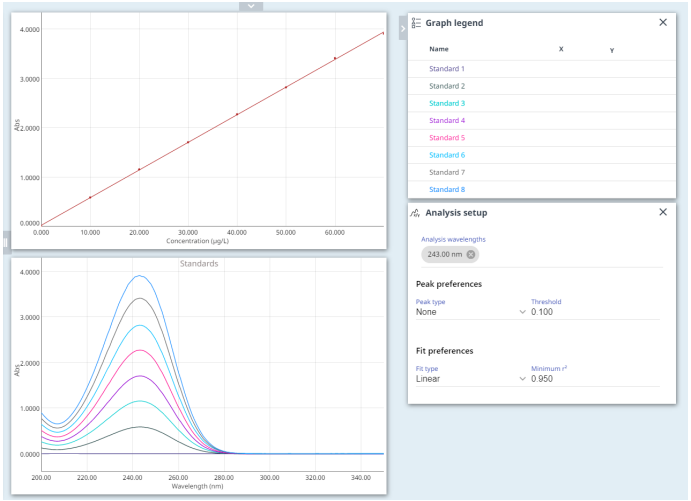

Figure 3. Wavelength scans of each of the standard solutions. The absorbance at 243 nm was used to create the calibration and subsequent quantitation of samples.

As the use of a Cary Sipper has the potential to be quicker than manually filling cuvettes, a series of measurements were done to quantify any time saving. 30 solutions (10 standards and 20 samples) were measured four different ways:

1. Without the Sipper, using a single 3.5 mL cuvette. The cuvette was manually filled, emptied, and rinsed for each measurement.

2. Without the Sipper, using three standard 3.5 mL cuvettes. The cuvettes were manually filled, emptied, and rinsed for each measurement. All three cuvettes were measured at the same time using the simultaneous measurement capabilities of the Cary 3500 Multicell instrument.
3. With the Sipper pumping to a single flow cell.
4. With the Sipper pumping to three flow cells.

The measurements were done using the instrument parameters shown in Table 4.

Table 3. Instrument parameters used for the quantitation measurements.

Parameter	Setting
Wavelength Range (nm)	200 – 350
Spectral Bandwidth (nm)	1
Averaging Time (s)	0.1
Data Interval (nm)	1
Flow Cell Volume (µL)	390
Fill Time (s)	30
Hold Time (s)	10
Rinse Time (s)	30

Table 4. Instrument parameters for the speed comparison measurements.

Parameter	Setting
Wavelength Range (nm)	200 – 350
Spectral Bandwidth (nm)	1
Averaging Time (s)	0.1
Data Interval (nm)	1
Flow Cell Volume (µL)	390
Fill Time (s)	15
Hold Time (s)	5
Rinse Time (s)	15

Results and discussion

Calibration linearity

The calibration curve generated from the eight standards using the Cary Sipper had an R^2 value of 0.9997 and the curve created from the cuvette measurements had an R^2 value of 0.9998. The Cary 3500 has excellent photometric linearity beyond 3 Abs which allows for highly concentrated liquid samples to be measured with photometrically accurate results.

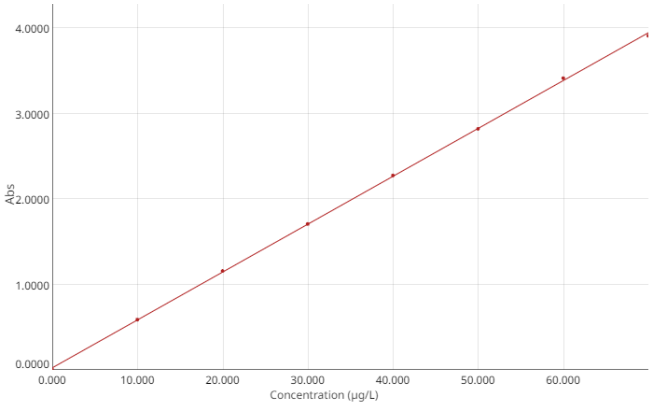

Figure 4. The calibration curve generated using manually filled quartz cuvettes.

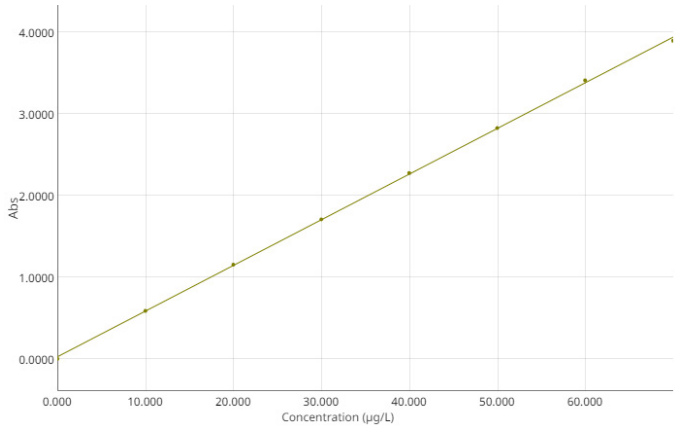

Figure 5. The calibration curve generated using the Cary Sipper.

Measurement precision

Each of the 20 sample solutions was measured three times on both instrument setups. When using the Cary Sipper, the sample solution was pumped into the flow cell, measured and then replaced with rinse solution, before the flow cell was again filled with the same sample solution. This was repeated three times for each of the 20 samples. When using the 3.5 mL cuvettes, the cuvette was filled with sample, measured and then rinsed before refilling with another aliquot of the same sample. This was repeated three times for each of the 20 samples.

As shown in Table 5, both sets of results had a high level of precision, with %RSD values well below the 2% typically specified by pharmacopeial methods. The %RSD across all six measurements was 0.1869%.

Table 5. The absorbance data from the measurement of the 20 samples, with each sample being measured three times on each instrument setup. The final %RSD column is the precision of all six measurements.

	Measurements With Sipper		Measurements With Manual Cuvettes		%RSD n=6
Sample	Mean n =3 (Abs)	%RSD	Mean n =3 (Abs)	%RSD	
1	2.2091	0.0775	2.2153	0.0197	0.1470
2	2.4895	0.3394	2.4763	0.0400	0.3313
3	3.8029	0.0462	3.7888	0.1150	0.1988
4	1.5972	0.1706	1.5912	0.0142	0.2119
5	0.7646	0.0991	0.7666	0.0190	0.1435
6	2.4438	0.0372	2.4353	0.0398	0.1766
7	3.0153	0.1773	3.0050	0.0315	0.1997
8	2.1405	0.2443	2.1333	0.0921	0.2277
9	3.6846	0.1805	3.6816	0.0161	0.1121
10	1.0538	0.1516	1.0581	0.0394	0.2241
11	2.4493	0.2714	2.4367	0.0441	0.3037
12	2.4525	0.2948	2.4351	0.0256	0.3941
13	0.8735	0.1588	0.8722	0.0330	0.1209
14	3.1695	0.2032	3.1676	0.0525	0.1248
15	2.7457	0.1754	2.7409	0.0389	0.1364
16	1.7695	0.2143	1.7631	0.0086	0.2186
17	2.0182	0.1966	2.0190	0.0207	0.1160
18	1.7909	0.1568	1.7863	0.1621	0.1818
19	2.3171	0.0425	2.3193	0.0129	0.0539
20	1.1099	0.0229	1.1098	0.1987	0.1157

Sample quantitation

The mean of the three absorbance readings of each sample and the calibration curve were used to determine the concentration of vitamin C in each sample solution, using the Beer Lambert law. This concentration was then used to calculate the weight of vitamin C in each tablet measured. The results are shown in Table 6. The average difference between the calculated weight and the label weight using the Sipper setup was 1.8%, with the difference ranging from 0.5 to 5.3%. The average difference for the same 20 samples, measured using the cuvettes, was 1.9%, with the difference ranging from 0.6 to 5.6%. The results were well within the ±10% acceptance criteria for ascorbic acid tablets, as specified in the USP (*1*), indicating that the tablets met their labelling requirements.

Table 6. The calculated concentrations of each sample, using the two instrument setups. The vitamin C content of each tablet was calculated from the sample concentrations and this was compared (% difference) to the stated label value of 1000 mg in each tablet.

	Sipper setup			Cuvette setup		
Sample no.	Measured Conc. (mg/L)	Calculated Vit C Content (mg)	Difference between calc. value and label value (%)	Measured Conc. (mg/L)	Calculated Vit C Content (mg)	Difference between calc. value and label value (%)
1	39.00	988.9	1.1	39.12	991.9	0.8
2	44.01	985.1	1.5	43.78	980.0	2.0
3	67.46	1021.3	2.1	67.22	1017.6	1.7
4	28.07	993.6	0.6	27.97	990.2	1.0
5	13.21	1019.7	1.9	13.25	1023.2	2.3
6	43.19	1017.2	1.7	43.05	1013.8	1.4
7	53.40	1023.9	2.3	53.22	1020.6	2.0
8	37.78	968.7	3.2	37.65	965.6	3.6
9	65.35	990.0	1.0	65.30	989.4	1.1
10	18.37	1028.9	2.8	18.46	1033.8	3.3
11	43.29	992.6	0.7	43.07	987.6	1.3
12	43.35	1018.4	1.8	43.05	1011.3	1.1
13	15.15	982.2	1.8	15.13	981.2	1.9
14	56.15	987.1	1.3	56.12	986.6	1.4
15	48.58	995.2	0.5	48.51	993.7	0.6
16	31.15	950.1	5.3	31.05	946.9	5.6
17	35.59	1004.6	0.5	35.62	1005.3	0.5
18	31.53	1028.7	2.8	31.46	1026.3	2.6
19	40.93	992.5	0.8	40.98	993.7	0.6
20	19.37	1024.1	2.4	19.38	1024.4	2.4

Measurement time

The Cary Sipper offered considerable time saving, compared to manually filling cuvettes. As shown in Table 7, using the Sipper with three flow cells reduced the time to measure 30 samples by 65%, compared to measuring the samples one by one using a standard cuvette. Without the Sipper, measuring three cuvettes at the same time offered a time saving of 24%, compared to filling, emptying and refilling a single cuvette three times. Using the Sipper with three flow cells was 54% quicker than manually using three cuvettes at the same time in a Cary 3500 Multicell instrument.

Table 7. The time taken to measure 30 samples using the four different instrument setups.

Mode of Operation	Measurement Time n = 30	Time Reduction (%) (Compared to manual handling with single cuvette)
Manual cuvette handling		
1. Compact module (1 sample cuvette)	21 min 30 s	
2. Multicell module (3 sample cuvettes)	16 min 26 s	24%
Using the Sipper Filling Time (15 s), Holding Time (5 s), Rinsing Time (15 s)		
3. 1 flow cell	19 min 32 s	9%
4. 3 flow cells	7 min 30 s	65%

Conclusion

This study compared the use of the Cary Sipper to pump solutions for measurement in a UV-Vis spectrophotometer, versus manually filling and emptying standard cuvettes. The Sipper proved to be as precise and 65% quicker than the manual measurements.

The absorbance of the samples measured using the two different setups were closely aligned. The %RSD across all six measurements was 0.1869, indicating a high level of precision.

The measurement of three samples using standard cuvettes proved to be 24% quicker when using the simultaneous measurement capabilities of the Cary 3500 UV-Vis spectrophotometer to measure all three at once.

The Sipper settings were controlled from the instrument software and could be stored as part of an instrument method. This allows consistent settings to be used for the analysis.

The Cary 3500 UV-Vis spectrophotometer, fitted with the Cary Sipper, proved to be an ideal instrument for the routine measurement of multiple liquid samples. It offers faster analysis time and considerable time savings for the workflow. The Cary 3500 high absorbance range reduces the need for sample dilution and it offers improved precision and accuracy over manually measuring samples using cuvettes.

References

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