Application Note

Biopharmaceutical/
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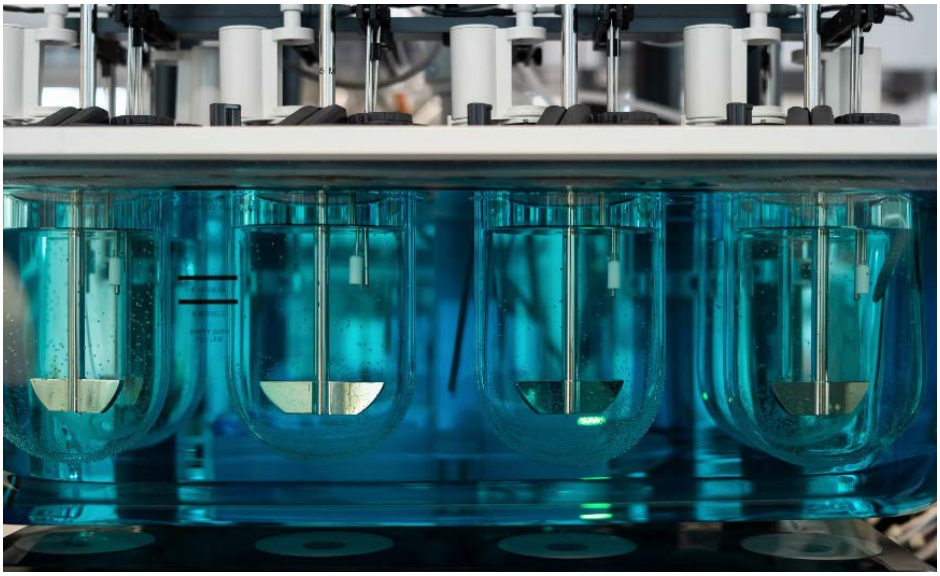
Automating Dissolution Testing with an Agilent Online LC System



Authors

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Abstract

The dissolution rate of a dosage form is a key analytical process to measure drug quality. During a dissolution test, the researcher must take multiple samples from six vessels of dissolution solution for replicate testing within a specified time period. The next step is to conduct intensive testing and analyses on the samples collected at different time points after the dissolution experiment is completed. The entire analysis is a cumbersome process and prone to human error. In this application note, an Agilent InfinityLab Online LC system was coupled with the Agilent Flexible Cube to enable fully automated, unattended, real-time dissolution testing. The automatic sample delivery and multiple sample point switching functions using the Flexible Cube can ensure the accuracy and efficiency of automated analysis.

Introduction

According to the definition in General Chapter 0931 of Pharmacopoeia of the People's Republic of China (2020 Edition), "dissolution rate refers to the rate and extent at which an active drug dissolves from ordinary dosage forms such as tablets, capsules or granules under specified conditions". Based on the results of a dissolution test, the release of the drug in the human body and the content of the pharmaceutical ingredient in the dosage form can be predicted.

In the conventional mode of dissolution testing, a researcher must take samples from six dissolution vessels in turn within a specified time for replicate tests. This sampling is done to obtain an effective dissolution curve, to analyze the dissolution rate, and to measure the extent of dissolution of the dosage form. The timeliness and accuracy of sampling has a direct impact on the test results. The source vessel of each sample and the time of sampling should be recorded in detail upon sampling. In addition, since the sampling points are numerous and dense, only after all samples have been collected, can the assay be uniformly conducted by HPLC. This test mode not only requires the cooperation of multiple operators but also fails to monitor the drug dissolution behavior in real time during the dissolution experiment. Meanwhile, the cumbersome sampling procedure and pretreatment steps may also introduce human errors.

In this application, with the Agilent Online LC system coupled with the Flexible Cube module, a fully automated analysis system for dissolution testing was established. The system uses the Agilent Online LC Monitoring software to monitor and trigger the Flexible Cube to perform the sampling from dissolution vessels at specified time points. Through flow path switching, the system regularly transfers the dissolution samples at each time point to the Online LC system for real-time analysis. Analysts can view the dissolution process or release rate in real time. The analysts can modify the pharmaceutical process or enable batch release, on the quality side, based on the real-time test results. This automation delivers actionable results in a timely manner.

Experimental

Reagents and samples

Acetonitrile: HPLC grade and purchased from Merck & Co., Inc (Darmstadt, Germany); experimental water: HPLC grade water from purification system, model Milli-Q (EMD Millipore, Billerica, MA, USA); sodium dihydrogen phosphate: HPLC grade, purchased from CNW; Phosphoric acid: HPLC grade, purchased from Dikma Technologies Inc. (CA, USA). Samples: Purchased sodium valproate tablets (immediate-release tablets/sustained-release tablets).

Instrumentation

An Agilent InfinityLab Online LC system consisting of the following Agilent modules was used: Agilent 1260 Infinity II Flexible Pump (part number G7104C); Agilent 1260 Infinity II Online Sample Manager Set (part number G3167AA); Agilent 1260 Infinity II Multicolumn Thermostat (part number G7116A), fitted with a standard heat exchanger (part number G7116-60051); and Agilent 1260 Infinity II Diode Array Detector (part number G7117C), fitted with a Max-Light cartridge flow cell (10 mm, part number G4212-60008).

While an Agilent 708-DS dissolution apparatus was running, an Agilent 1290 Infinity Flexible Cube (part number G4227A) was used for sample transfer between the dissolution apparatus and the Online LC system. The Flexible Cube module is shown in Figure 1. It includes a reciprocating single-cylinder piston pump and a 12 ps/13 pt Stream Selector, Quick Change Valve (part number G4235A). The valve position, pumping time, and pumping rate can be controlled via the chromatography data system (CDS) software.

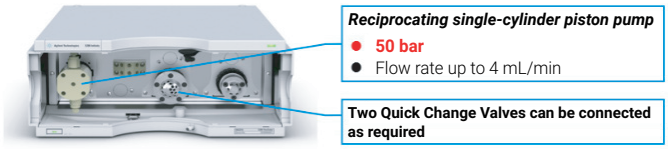


Figure 1. Agilent 1290 Infinity Flexible Cube module.

A schematic of the instrument connections in this experiment is illustrated in Figure 2. The Flexible Cube module connects the Online LC system and the dissolution apparatus. Six sampling cannulas are fixed onto the multifunctional sampling rack at the end of the dissolution apparatus. A full-pass filter (Agilent full flow filter, part number 17-4003) is installed at the front end of each sampling cannula. The other end of the sampling cannula is connected to a Quick Change Valve of the Flexible Cube, through a line. The Quick Change Valve 1 - 6 corresponds to dissolution vessels 1 - 6, respectively. Then the Flexible Cube outlet is connected to the external valve of the Online LC system via tubing to complete the connection between the dissolution apparatus and the Online LC system.

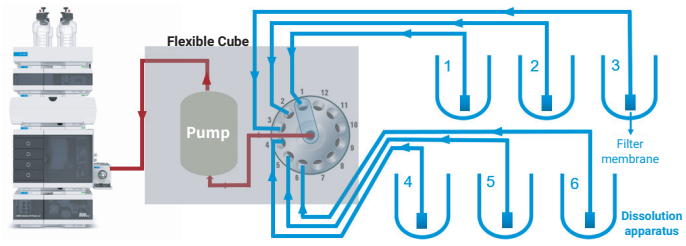


Figure 2. Schematic of instrument connections.

For software, Agilent OpenLab CDS version 2.7 and Agilent Online LC Monitoring software version 1.1 were used.

Standard preparation

A desired amount of sodium valproate standard was taken and diluted with purified water to obtain a standard solution with a sodium valproate content of 0.3 mg/mL.

Development of LC conditions and related experiment

This dissolution experiment was conducted on sodium valproate tablets of different specifications. Before the linear dissolution test, the sodium valproate standard solution was used first to develop a rapid HPLC method to clarify the chromatographic conditions.

Column	Poroshell EC C18 3.0 mm × 50 mm, 2.7 µm, part number 699975-302
Column Temperature	45 °C
Wavelength	210 nm
Injection Volume	20 µL
Flow Rate	2.0 mL/min
Mobile Phase	10 mM sodium dihydrogen phosphate pH 2.0: acetonitrile, 60:40
Run Time	1.0 min
Sodium Valproate Standard Solution	A desired amount of sodium valproate standard was taken and diluted with purified water to obtain a solution with a sodium valproate content of 0.3 mg/mL.

Chromatographic injection method

Under the proposed chromatographic conditions, the sodium valproate standard solution was injected and analyzed to obtain a chromatogram. As shown in Figure 3, the substance of interest, i.e., sodium valproate, has a good peak shape. The retention time of the main peak was 0.382 minute, thus the time interval between single injections could be shortened to within 1 minute.

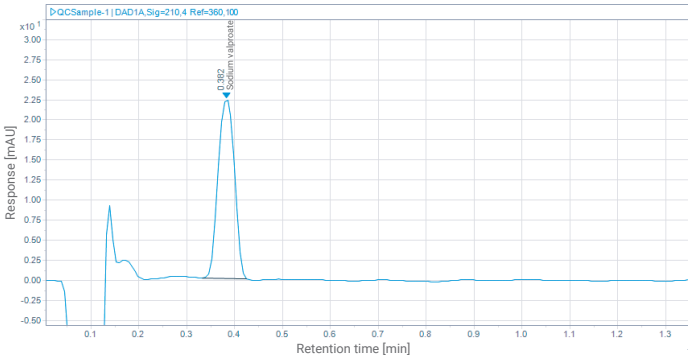


Figure 3. HPLC chromatogram of sodium valproate standard solution under test.

Tubing test

In this experiment, as a sample delivery unit, the Flexible Cube was integrated into the Online LC system. The Quick Change Valve of the Flexible Cube was used to switch between flow paths from the different dissolution vessels. Then the single-piston pump was used to complete the flow path substitution with content taken from the previous dissolution vessel to another/next one. The effects of the dead volume of the connection and possible liquid diffusion in the tubing were examined when determining the test accuracy. The time required for solution delivery was investigated before implementing the dissolution experiment.

The entire flow path was fully flushed with acetonitrile, and the tubing was placed in the prepared sodium valproate standard solution. With the flow rate in the Flexible Cube set to 4.0 mL/min, the solution was collected into an injection vial, which was changed once every 7.5 s (0.5 mL of solution per vial was collected). The solutions within 7.5 to 150 seconds were consecutively collected and the collected samples were analyzed, one-by-one, under the previously stated chromatographic conditions. The changes in the area of the sodium valproate peak, with sampling time, were analyzed to calculate the accuracy of the target compound content. These results were plotted to a trend chart of accuracy versus sampling time. Table 1 and Figure 4 show the results. As the sampling time gradually increased, the sodium valproate content in the tubing tended to increase, and after 82.5 seconds, the content accuracy values were greater than 99.5% and remained stable. It can be considered that the liquid collected accurately reflected the concentration of the target compound. Therefore, after each tubing switch, the flushing time should be as close to 82.5 seconds (1.375 minutes) as possible. This flushing ensures that the dissolution solution can fully replace the original solution in the tubing, thereby providing accurate dissolution test results.

Table 1. Trend of peak area of the constituent of interest.

Sampling Time (seconds)	Peak Area	Accuracy
7.5	0	0.00%
15.0	0	0.00%
22.5	2.806	3.16%
30.0	27.025	30.41%
37.5	59.359	66.79%
45.0	79.404	89.34%
52.5	85.623	96.34%
60.0	87.235	98.15%
67.5	87.969	98.98%
75.0	88.299	99.35%
82.5	88.522	99.60%
90.0	88.691	99.79%
97.5	88.742	99.85%
105.0	88.832	99.95%
112.5	88.935	100.06%
120.0	88.899	100.02%
127.5	89.030	100.17%
135.0	88.957	100.09%
142.5	89.204	100.37%
150.0	88.915	100.04%

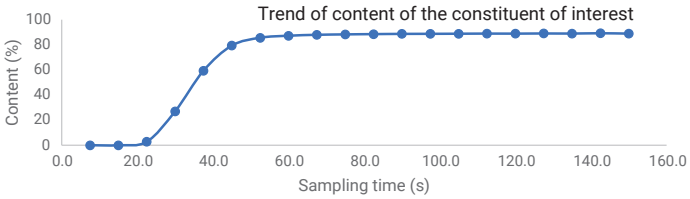


Figure 4. Trend chart of content accuracy of the constituent of interest.

Experimental settings of online dissolution test

Dissolution testing is an important means to evaluate the rate and extent of drug release from oral solid dosage forms. The drug dissolution process consists of an initial rapid release stage, a subsequent slow release stage, and a final stabilization stage. Therefore, selection of an appropriate sampling frequency based on drug properties and dosage form is crucial to the accuracy of dissolution testing.

Sustained-release tablets and immediate-release tablets are considered in the method. It normally takes a few hours for a sustained-release tablet to be fully released in vivo. Usually, the first sample is collected at around 1 hour to evaluate the initial release rate of the drug. Then a sampling time point can be set every 1 or 2 hours, depending on the drug release profile, until the drug is completely released. Such a setup can provide a detailed profile of drug release and ensure that the expected percentage of the drug is dissolved within a specified time. In conventional dissolution experiments, at each sampling time point, sample collection, treatment and delivery must be done manually. The sampling of sustained-release tablets may last for 24 hours or longer. The prolonged and exhausting process by the operators may reduce the sampling accuracy and increase the risk of operational errors. Unlike sustained-release tablets, immediate-release tablets are designed to release the drug quickly, usually releasing most of the drug in 30 minutes. The first sampling time point is set to a time within 5 minutes to evaluate the initial release rate of the drug. Thereafter, multiple sampling time points were set within 5 to 30 minutes to capture the critical stage of drug release. Manual procedures during high-frequency sample collection usually face tougher challenges, especially in terms of sampling accuracy and consistency. Owing to the rapid dissolution of immediate-release tablets, errors in sampling time and differences in sample taking behavior among different operators may result in missing the critical stage of rapid drug release.

Combining the Online LC system with the Flexible Cube enables fully automated sampling and dissolution testing, significantly improving experimental efficiency and data quality while increasing data accuracy.

This experiment was conducted to test the feasibility and accuracy of a method using the Online LC system with the Flexible Cube, with sodium valproate sustained-release tablets and sodium valproate immediate-release tablets as examples.

Results and discussion

Dissolution test of sodium valproate sustained-release tablets

Based on the release profile of sodium valproate sustained-release tablets, four sampling time points were set in this experiment and 6 sets of dissolution tests were conducted simultaneously. The sampling schedule is detailed in Table 2.

Table 2. Time schedule of dissolution test of sodium valproate sustained-release tablets.

Time (minutes)	Time Point 1	Time Point 2	Time Point 3	Time Point 4
Vessel 1	01h00m	03h00m	06h00m	12h00m
Vessel 2	01h05m	03h05m	06h05m	12h05m
Vessel 3	01h10m	03h10m	06h10m	12h10m
Vessel 4	01h15m	03h15m	06h15m	12h15m
Vessel 5	01h20m	03h20m	06h20m	12h20m
Vessel 6	01h25m	03h25m	06h25m	12h25m

Based on the sampling times shown in Table 2, the minimum sampling interval in the dissolution experiment of sodium valproate sustained-release tablets was 5 minutes. The tubing cleaning and sampling path content solution substitution can be completed, followed by real-time dissolution analysis using an Online LC system. For example, the experimental schedule for the first three sampling time points is detailed in Figure 5.

Analysis Procedure for Sodium Valproate Sustained-Release Tablets	1 hour	1 hour 5 minute	1 hour 10 minute
Flexible Cube	Use vessel 1 to flush the tubing	Use vessel 2 to flush the tubing	Use vessel 3 to flush the tubing
LC system	Analyze the sample in vessel 1	Analyze the sample in vessel 2	Analyze the sample in vessel 3

Figure 5. Analysis procedures for sodium valproate sustained-release tablets at the first three sampling time points.

There is a long interval between the sampling time of the last vessel for the first time point setting (01h25m) and the sampling time of the first vessel for the second time point setting (03h00m). The Online LC Monitoring software can be used to set “sleep method” to allow the LC system to run at a lower flow rate within idle time frames. Then the software can set a “wake-up method” to equilibrate the chromatographic system prior to injection for data acquisition. The settings are detailed in Figure 6.

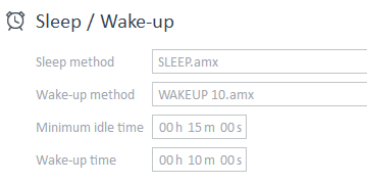
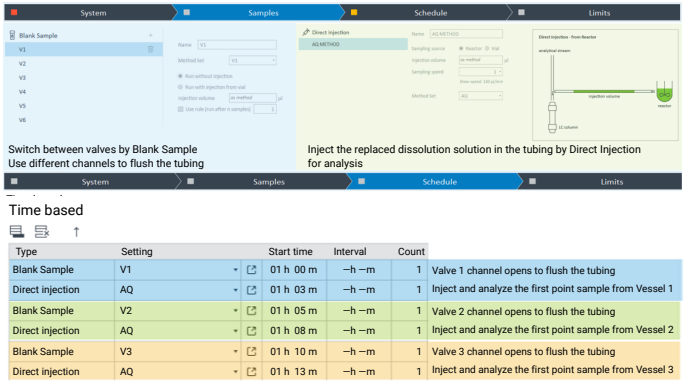


Figure 6. Settings of sleep/wake-up method in Online LC Monitoring software.

After the CDS acquisition method were set, the sample collection method, test method, and specific sampling time points were set in the Online LC Monitoring software, as detailed in Figure 7. In this experiment, the method for sample collection from the dissolution vessel was set to "direct injection", as detailed in Figure 8. In different application scenarios, analysts can also use the Online LC Monitoring software to set other sample collection methods. For example, samples can be collected into vials while data is being acquired, or the eluate can be diluted prior to injection.



Type	Setting	Start time	Interval	Count	
Blank Sample	V1	01 h 00 m	–h –m	1	Valve 1 channel opens to flush the tubing
Direct injection	AQ	01 h 03 m	–h –m	1	Inject and analyze the first point sample from Vessel 1
Blank Sample	V2	01 h 05 m	–h –m	1	Valve 2 channel opens to flush the tubing
Direct injection	AQ	01 h 08 m	–h –m	1	Inject and analyze the first point sample from Vessel 2
Blank Sample	V3	01 h 10 m	–h –m	1	Valve 3 channel opens to flush the tubing
Direct injection	AQ	01 h 13 m	–h –m	1	Inject and analyze the first point sample from Vessel 3

Figure 7. Setting acquisition method, sampling method, and trigger time in Online LC Monitoring software.

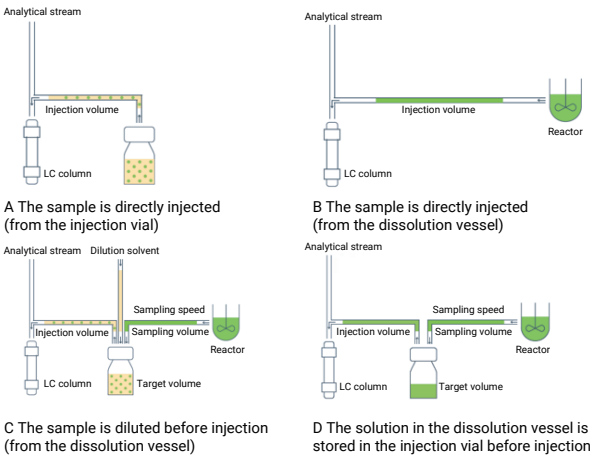


Figure 8. Different modes of sampling and analysis for the Online LC system.

After completing the preceding settings, the operator should ensure that the dissolution apparatus and the Online LC system start running synchronously. Next, set the automatic dosing interval for the dissolution apparatus to 5 minutes to complete the dissolution testing of sodium valproate sustained-release tablets and data processing will start automatically.

The dissolution test of sodium valproate sustained-release tablets was carried out under the conditions described in the experimental section. The Online LC Monitoring software was used to automatically process the collected data by the preset processing method, followed by statistical data analysis, and dissolution curve plotting.

Table 3. Statistics of peak areas at various sampling time points for sodium valproate sustained-release tablets.

Test Time (hour)	Vessel 1	Vessel 2	Vessel 3	Vessel 4	Vessel 5	Vessel 6	Mean	RSD
1	33.098	34.539	34.087	32.559	32.657	33.452	33.399	2.4%
3	62.096	63.112	62.433	63.559	65.398	63.699	63.383	1.8%
6	94.639	96.89	95.585	96.249	95.306	94.282	95.492	1.0%
12	114.753	116.493	115.281	114.416	112.306	113.073	114.387	1.3%

Table 4. Results of dissolution rates of sodium valproate sustained-release tablets.

Test Time (hour)	Vessel 1	Vessel 2	Vessel 3	Vessel 4	Vessel 5	Vessel 6	Mean
1	23.9%	24.9%	24.6%	23.5%	23.6%	24.2%	24.1%
3	44.9%	45.6%	45.1%	45.9%	47.2%	46.0%	45.8%
6	68.4%	70.0%	69.0%	69.5%	68.8%	68.1%	69.0%
12	82.9%	84.1%	83.3%	82.6%	81.1%	81.7%	82.6%

The results of 6 replicate samples collected at the same sampling time point were compared. The relative standard deviation (RSD) of the main peak area was calculated to evaluate the reproducibility of the test. These results are tabulated in Table 3. As can be seen from the table, the RSD value of the peak area of the compound of interest obtained from 6 replicate experiments, with a sampling time of 1 hour, was 2.4%. This result was less than 20% as required by the method. The RSD values of the results obtained from the replicate experiments with sampling times of 3, 6, and 12 hours were 1.8, 1.0, and 1.3%, respectively. All of these values were less than 10%, as required by the method. These results indicate that the method has good reproducibility under the proposed test conditions and can meet the dissolution testing requirements.

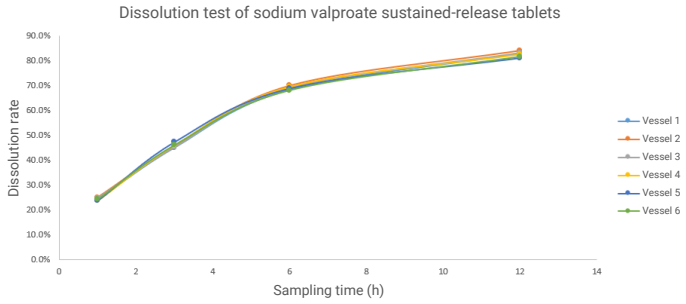


Figure 9. Dissolution curves of sodium valproate sustained-release tablets.

Each dissolution curve of sodium valproate sustained-release tablets was plotted after comparing the peak area with the standard solution, and the results are shown in Figure 9. Based on the data listed in Table 4, the mean dissolution rates at dissolution times of 1, 3, 6, and 12 hours were 24.9, 47.3, 71.2, and 85.3%, respectively. This data indicates that eventually the drug was dissolved within the set experimental period.

Dissolution test of sodium valproate immediate-release tablets

Different from the sustained-release tablets, more frequent sampling time points for dissolution testing were set based on the release profile of the sodium valproate immediate-release tablets. Specifically, four sampling time points were set and six dissolution vessels were assayed simultaneously, as detailed in Table 5.

Table 5. Time schedule of dissolution test of sodium valproate immediate-release tablets.

Time (minute)	Time Point 1	Time Point 2	Time Point 3	Time Point 4
Vessel 1	3	15	30	45
Vessel 2	5	17	32	47
Vessel 3	7	19	34	49
Vessel 4	9	21	36	51
Vessel 5	11	23	38	53
Vessel 6	13	25	40	55

According to the sampling schedule shown in Table 5, the shortest time interval between two sampling time points was 2 minutes. After each change of the dissolution vessel, the tubing cleaning and flow path content substitution time was 1.3 minutes, while it took 1 minute to complete a single injection analysis. If tubing cleaning preceded sample analysis, the total duration of a single run of dissolution solution delivery and testing would be greater than 2 minutes, failing to meet the minimum time interval requirement. Therefore, synchronizing sample delivery with sample collection was considered. Taking the first three sampling time points (3, 5, and 7 minutes) in Table 5, the experimental schedule is shown in Figure 10.

Analysis procedure for immediate-release tablets	3 minutes	5 minutes	7 minutes
Flexible Cube	Use Vessel 1 to flush the tubing	Use Vessel 2 to flush the tubing	Use Vessel 3 to flush the tubing
LC system	–	Analyze the sample in Vessel 1	Analyze the sample in Vessel 2

Figure 10. Tubing cleaning and analysis procedure for sodium valproate immediate-release tablets.*

* To achieve more frequent sampling and testing of immediate-release tablets, the experimental procedure for sustained-release tablets (sampling followed by analysis) was modified. Thanks to the flexibility of the Flexible Cube module, the tubing flushing in the Flexible Cube and the injection, and analysis in the Online LC system could be set up separately during the experiment. This flexibility enabled simultaneous ability of tubing flushing for the next injection and sample testing for the last injection.

After the CDS acquisition method was set, the operator set the sample collection method, test method, and specific sampling time points in the Online LC Monitoring software (Figure 11). After completing all settings, the dissolution apparatus and the Online LC system were set to start running synchronously. Then the dosing interval was set for the dissolution apparatus at 2 minutes to complete the dissolution testing of sodium valproate immediate-release tablets and data processing in 1 hour.

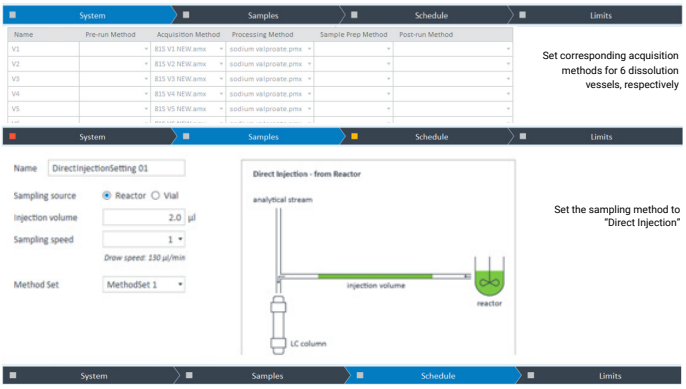


Figure 11. Setting acquisition method, sampling method, and trigger time in Online LC Monitoring software.

The dissolution test of sodium valproate immediate-release tablets was carried out under the conditions described in the experimental section. After the experiment was done, the Online LC Monitoring software was used to automatically process the collected data by the preset processing method, followed by statistical data analysis and dissolution curve plotting.

The peak areas of the compound of interest, in 6 replicate samples collected at the same sampling time point, were compared. The peak area RSD was calculated to evaluate the reproducibility of the test (Table 6). As can be seen from the table, the RSD value of peak area of the compound of interest obtained from 6 replicate experiments, with a sampling time of 3 minutes, was 1.5%, less than 20% as required by the method. The RSD values of the results obtained from the replicate experiments with sampling times of 15, 30, and 45 minutes were 0.6, 1.0, and 1.2%, respectively. And all of these RSD values were less than 10% as required by the method. These results indicate that the method has good reproducibility under the proposed test conditions and can meet the dissolution testing requirements.

Table 6. Statistics of peak areas in dissolution test of sodium valproate immediate-release tablets.

Test Time (minute)	Vessel 1	Vessel 2	Vessel 3	Vessel 4	Vessel 5	Vessel 6	Mean	RSD
3	23.40	22.80	22.69	22.76	23.16	22.48	22.88	1.5%
15	53.76	53.87	53.54	54.49	53.93	53.69	53.88	0.6%
30	54.80	54.79	54.18	54.48	53.43	53.86	54.26	1.0%
45	54.85	54.96	54.72	55.36	53.53	54.33	54.62	1.2%

Table 7. Results of dissolution rates of sodium valproate immediate-release tablets

Test Time (minute)	Vessel 1	Vessel 2	Vessel 3	Vessel 4	Vessel 5	Vessel 6	Mean
3	42.6%	41.5%	41.3%	41.4%	42.2%	40.9%	41.7%
15	97.9%	98.1%	97.5%	99.2%	98.2%	97.8%	98.1%
30	99.8%	99.8%	98.7%	99.2%	97.3%	98.1%	98.8%
45	99.9%	100.1%	99.6%	100.8%	97.5%	98.9%	99.5%

Each dissolution curve of sodium valproate immediate-release tablets was plotted after comparing the peak area with that of the standard solution, and the results are shown in Figure 12. Based on the data listed in Table 7, the mean dissolution rate at dissolution time of 3 minutes was 41.7% and the mean dissolution rate at dissolution time of 15 minutes was 98.1%. At dissolution times of 30 to 45 minutes, the dissolution rates remained stable and above 98%, indicating that, eventually, the drug had been dissolved within the set experimental time.

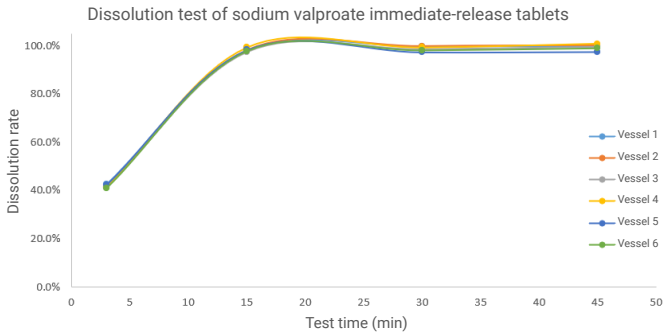


Figure 12. Plot of dissolution rates of sodium valproate immediate-release tablets.

Conclusion

In this application note, an Online LC system was coupled with the Flexible Cube module to offer one fully automated dissolution testing workflow. The CDS software was used to control the Flexible Cube to complete the flow path selection and dissolution solution delivery within the specified time limits.

The Online LC system was used to perform real-time testing of the collected dissolution solution from both the sustained-release and immediate-release tablets. The combination method showed good flexibility, efficiency, and improved data quality.

The use of the Agilent Online LC system coupled with the Agilent Flexible Cube module enabled an optimized experimental procedure, reduced manual operations, and improved test accuracy and efficiency. This developed method showed scalability and versatility, making it suitable for other experimental scenarios such as replicate reaction monitoring. Thus, the system has broad application prospects in drug development and manufacturing.

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