SHIMADZU
Excellence in Science

Application News

Ultra High Performance Liquid Chromatograph Nexera™ FV

USP-Compliant Online Dissolution Testing of Antipyretic Analgesic: Automatic Addition of Internal Standard

K. Matsuoka, A. Tanabe

User Benefits

- Dissolution testing can be automated.
- The automatic sampling function enables testing with good reproducibility.
- The automatic dilution and the automatic addition of internal standard improve work efficiency.

■ Introduction

Dissolution testing is conducted for formulation development, quality control, bioequivalence tests of generic drugs, etc.. In dissolution testing, the dissolution properties of a drug product are checked under specific conditions for certain periods. Dissolution testing takes a lot of labor and time, since dissolution media must be sampled from multiple vessels every sampling time and analyzed.

Nexera FV is an HPLC system for online dissolution testing. It can automate the processes from sampling dissolution media, HPLC analysis, up to report output. By automating tasks that used to be conducted manually by operators, the system realizes labor saving and throughput improvement. In addition, automating tasks prevent human errors from whole processes.

This article introduces an example of automatic addition of the internal standard (ISTD) to the dissolution medium and a USP-compliant online dissolution testing of an antipyretic analgesic tablet using the Nexera FV. For additional information on online dissolution testing, please refer to Application News 01-00029 and 01-00031.

■ Online Dissolution Testing with Nexera FV

Fig. 1 shows a comparative example of the workflow of conventional method and online dissolution testing with Nexera FV. The Nexera FV system can automate sampling of dissolution medium at designated times, filtration, dilution, addition of internal standards, HPLC analysis, and report generation processes which had been conducted manually.

Furthermore, the HPLC analytical conditions can be set easily by the dedicated software DT-Solution (Fig. 2), and a report summarizing multiple data such as the dissolution rate is prepared simultaneously with completion of the analysis (Fig. 3).

Nexera FV has two modes of analysis: direct injection mode to inject the dissolution media delivered from the dissolution tester directly to the HPLC, and fraction analysis mode to fractionate the dissolution media into vials and analyze them. The former is effective when an analysis is enough short to complete by the next sampling time. The latter is used in tests with short sampling intervals, and in cases where dilution or addition of internal standards is required.

As described in this article, use of the Nexera FV fraction analysis mode to automatically add the ISTD made it possible to achieve high efficiency in work that had been done manually in the conventional process.

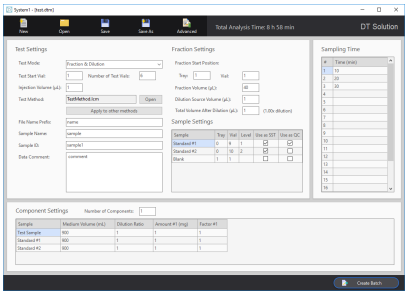

Fig. 2 DT-Solution Setting Window

Fig. 3 Multi-Data Report*1 Windows

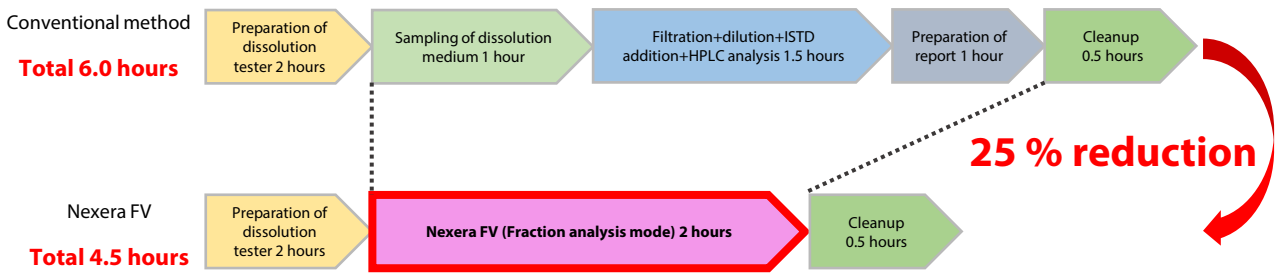

Fig. 1 Comparison of Workflow of Online Dissolution Testing*2

*1 Multi-Data Report is an optional function of LabSolutions™DB/CS to generate reports automatically.
*2 The time above is an example of an antipyretic analgesic with a dissolution test of 1 hour and an HPLC analysis of 0.5 hours (6 analyses of 6 minutes each).

■ Addition of ISTD Using Sample Preparation Function

In this article, the acetaminophen, aspirin, and caffeine in an antipyretic analgesic tablet were determined using the internal standard method. The sample preparation function of a Shimadzu SIL-30ACFV autosampler was used to add the ISTD to the standard sample and the fractions of dissolution medium. Fig. 5 shows the flow of ISTD addition using the autosampler, and Table 1 shows the sample preparation program. Fig. 4 shows an example of the vial arrangement in the autosampler when the sample preparation program in Table 1 is carried out. The ISTD vial (❶) is set in the control vial rack, and the flow vials which receive the dissolution media from the dissolution tester are set in the flow vial rack before starting the test. As dissolution medium fractionation vials (❷) and dilution/ISTD addition vials (❸), empty vials are set at the positions shown in Fig. 4. When the test is started and the specified time is reached, (1) the dissolution media from the dissolution tester are pumped to the flow vials, and (2) the dissolution media are fractionated to the dissolution medium fractionation vials (❷) by the autosampler. Following this, (3) the specified amounts of the ISTD in the ISTD vial (❶) and the fractionated dissolution medium in the fractionation vials (❷) are suctioned with a needle, and are then discharged together with the diluent into the dilution/ISTD addition vials (❸) and mixed. Finally, (4) 10 µL of the dissolution medium with addition of the prepared diluted ISTD by (3) is injected from the dilution/ISTD addition vials (❸) into the injection port (❹). These procedures of (❸),(❹) are carried out for all fractions. The ISTD used here was a methanol solution (before addition 3600 mg/L) of benzoic acid, and the diluent was methanol/glacial acetic acid = 95 : 5.

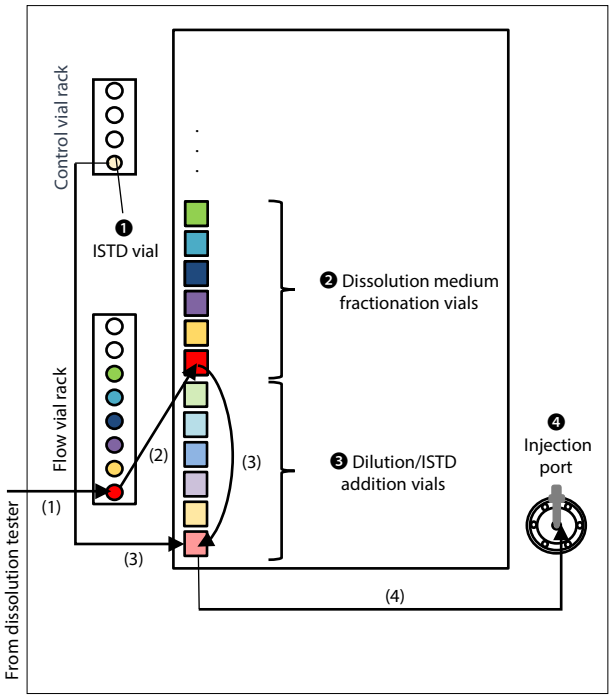

Fig. 4 Example of Vial Arrangements in Autosampler

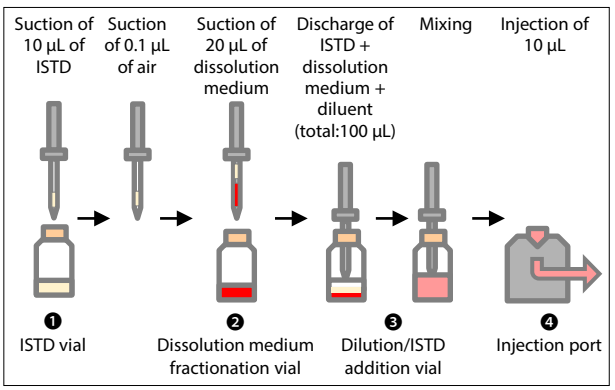

Fig. 5 Flow of Internal Standard (ISTD) Addition by Autosampler

Table 1 Sample Preparation Program

1	n.drain
2	disp 600,rs
3	vial.n 0,9
4	n.strk 52
5	aspir 10,ss
6	air.a 0.1,ss
7	d.rinse
8	a0=sn+6
9	vial.n 1,a0
10	n.strk ns
11	aspir 20,ss
12	vial.n rn,sn

13	n.strk ns
14	disp 100,ss
15	mix 3,5,45,2,10
16	n.drain
17	disp 600,rs
18	d.rinse
19	inj.p
20	v.inj
21	wait 2.0
22	goto f0
23	end

■ System Suitability Test

Table 2 shows the analytical conditions, and Fig. 6 shows the chromatogram of the mixed standard sample of acetaminophen, acetylsalicylic acid, and caffeine. It is thought that the sample also contained a small amount of salicylic acid which was formed by hydrolysis of the acetylsalicylic acid, and this appeared as a peak.

Table 3 shows the result of the system suitability test. The test conditions are described in the United States Pharmacopeia USP43-NF38 Acetaminophen, Aspirin, and Caffeine Tablets DISSOLUTION[(1)]. Table 4 shows the requirements of the system suitability test in USP43-NF38. System suitability was confirmed by the repeated analyses (n=6) of a 100 mg/L solution under the conditions shown in Table 2. Both the performance and the reproducibility of the system satisfied the requirements of USP43-NF38.

Table 2 HPLC Conditions

Column	Shim-pack™ Scepter C18-120*1 (100 mm × 4.6 mm I.D., 5 µm)
Mobile phase	Methanol / Glacial Acetic Acid / Water = 28 : 3 : 69
Flow rate	2 mL/min
Column temp.	45 °C
Injection vol.	10 µL
Vial	Shimadzu Vial, LC, 1.1 mL, Glass*2 Shimadzu Vial, LC *3
Detection	UV 275 nm

*1 P/N : 227-31020-04 *2 P/N : 228-21283-91
*3 P/N : 228-31600-91

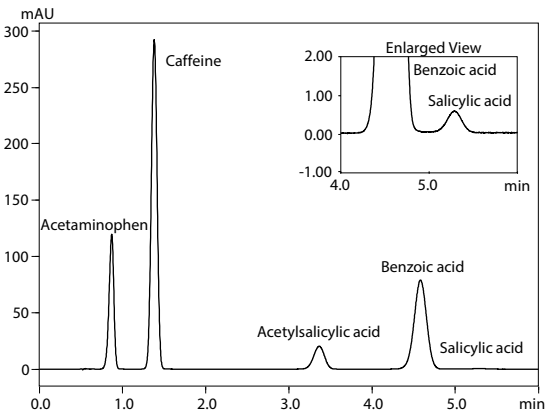

Fig. 6 Chromatograms of Mixed Standard (each 100 mg/L) of Acetaminophen, Acetylsalicylic Acid and Caffeine

Table 3 Results of System Suitability Test (each 100 mg/L)

Suitability requirements		Target Compound			Internal Standard	Judgement
		Acetaminophen	Caffeine	Acetylsalicylic acid	Benzoic Acid	
Resolution (n=6)	≥1.4	19.32	15.73	4.66		PASSED
Tailing factor (n=6)	≤1.2	0.87	0.98	0.94	0.97	PASSED
Retention Time (%RSD)	≤2.0	0.08	0.07	0.28	0.17	PASSED
Area (%RSD)	≤2.0	0.36	0.38	0.35	0.44	PASSED

Table 4 Requirements of System Suitability Test (USP43-NF38)

Resolution	≥1.4 between any of the analyte and ISTD
Tailing factor	≤1.2 for each analyte peak
Relative standard deviation	≤2.0 %

■ Dissolution Test

Fig. 7 shows the chromatogram of the dissolution medium from the commercially-available antipyretic analgesic tablet. Table 5 shows the dissolution conditions. The HPLC analytical conditions are the same as that shown in Table 2. The dissolution media were sampled automatically and filtered at the specified time, the ISTD was added automatically by using the Nexera FV, and then analyzed.

Table 6 shows the dissolution rates of each component at a dissolution time of 60 min. USP43-NF38 Acetaminophen, Aspirin, and Caffeine Tablets DISSOLUTION requires dissolution of 75 % of the label contents of each of the three components acetaminophen, acetylsalicylic acid, and caffeine within the dissolution time of 60 min. In this test, the results were within the tolerance required in the USP, as dissolution rates of 75 % or more were achieved in the dissolution time of 60 min with all components and all vessels.

Table 5 Dissolution Conditions

System	NTR-6600AST (TOYAMA SANGYO CO., LTD.)
Dissolution method	Paddle
Dissolution media	Pure water
Media volume	900 mL
Rotation speed	100 rpm
Bath temperature	37 ˚C
Total time	60 min
Sampling time	60 min

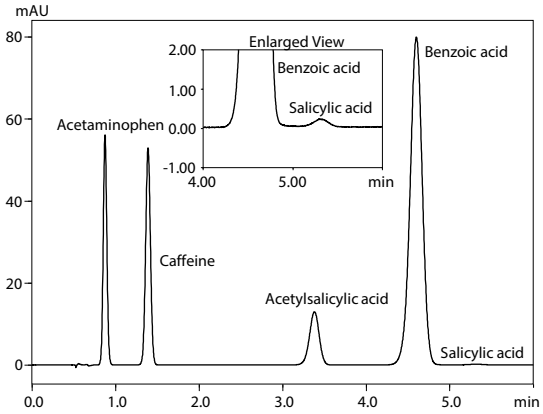

Fig. 7 Chromatograms of Antipyretic Analgesic (Dissolution Time : 60 min)

Table 6 Dissolution Rates* in Dissolution Test of Antipyretic Analgesic Tablets (Dissolution Time : 60 min, %)

Compound / Vessel No.	Acetaminophen	Caffeine	Acetylsalicylic acid
1	109.3	123.1	104.6
2	106.4	121.5	109.7
3	107.7	122.9	105.6
4	108.9	119.3	103.7
5	113.2	131.8	102.8
6	110.7	121.2	103.2
Judgement	PASSED	PASSED	PASSED

* Dissolution rate (%) = Concentration (mg/L) × Media volume 0.9 (L) / Labeled amount (mg) × 100

■ Conclusion

A USP-compliant online dissolution test was carried out using a commercially-available antipyretic analgesic tablet containing acetaminophen, acetylsalicylic acid, and caffeine. In comparison with the conventional method, a large reduction in work time and increase in work efficiency were achieved by automatic dilution and addition of the internal standard using the Nexera FV. As a result, it was found that the time required for the total process can be reduced by approximately 25 %.

<Reference>
(1) USP43-NF38-49 "Acetaminophen, Aspirin, and Caffeine Tablets DISSOLUTION"

01-00030-EN First Edition: Mar. 2022

SHIMADZU
Shimadzu Corporation
Analytical & Measuring Instruments Division
Global Application Development Center

www.shimadzu.com/an/