Application note | 000461

UHPLC

Contraceptive dissolution testing by liquid chromatography using large volume injections

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Keywords

Vanquish Flex UHPLC, injection volume, contraceptives, dissolution testing, loop, drospirenone, ethinylestradiol, large volume injection, USP

Application benefits

- Optional large volume injection loops and multi-draw operation enable Thermo Scientific™ Vanquish™ Flex and Horizon samplers for a wide range of injection volumes
- Highly reproducible results for low and high injection volumes

Goal

Dissolution testing of drospirenone and ethinylestradiol tablets according to the USP monograph.

Introduction

Drug dissolution testing is a standard procedure in the pharmaceutical industry, playing important roles in drug development stages as well as in quality control. Very often, dissolution results are critical in batch-to-batch comparisons and product release decisions. The conditions of these in vitro tests are designed to simulate—more or less closely—the in vivo conditions in the human body after consumption to provide reliable drug release information. Usually larger volumes (up to 1 L) of mainly aqueous media are utilized to dissolve one dose of the product. The resulting high dilution factor of the active pharmaceutical ingredients must be considered regarding the sensitivity during analytical detection, especially when low dosage forms are tested. Oral contraceptive pills are one good example for such low dosage products, as modern products (so called micro-pills) contain far lower amounts of active ingredients than previous generations.

The United States Pharmacopeia (USP) provides instructions for the dissolution testing of a variety of formulations including the description of dissolution test apparatuses. Contraceptive ingredients are usually analyzed by high performance liquid chromatography (HPLC) with optical UV and/or fluorescence detection (FLD). To compensate for the strong dilution by the dissolution media, some tests require exceptionally high injection volumes to provide sufficient assay sensitivity. This application note showcases the implementation of the USP dissolution test 2 for tablets labeled to contain 3 mg of drospirenone (D) and 0.02 mg of ethinylestradiol (EE), which implies the injection of 200 µL for analysis by HPLC-UV-FLD.[1]

Experimental

Chemicals

Chemical name	Part number
Deionized water, 18.2 MΩ·cm resistivity or higher	N/A
Acetonitrile, Optima™ LC/MS grade, Fisher Chemical™	A955-212
Methanol, Optima™ LC/MS grade, Fisher Chemical™	A456-212
Drospirenone CRS (batch 2)	Y0001105 (EDQM)[2]
Ethinylestradiol CRS (batch 5)	E1900000 (EDQM)[2]

Sample handling

Item name	Part number
Fisherbrand™ Isotemp™ Hot Plate Stirrer	SP88857205
Fisherbrand™ Mini Centrifuge	12-006-901
Thermo Scientific™ SureStop™ 9 mm Wide Opening Clear Vial Convenience Kit	2-SVWGKST-CPK

Instrumentation

Module	Part number
Thermo Scientific™ Vanquish™ Flex UHPLC system consisting of:	
System Base Vanquish Horizon/Flex	VF-S01-A-02
Vanquish Binary Pump F	VF-P10-A-01
Vanquish Split Sampler FT*	VF-A10-A-02
Either equipped with Sample Loop 250 µL, MP35N or Sample Loop 1000 µL, MP35N	6850.1970 6850.1980
Vanquish Column Compartment H	VH-C10-A-03
Vanquish Diode Array Detector FG	VF-D11-A-01
Semi-micro flow cell, biocompatible MP35N, 7 mm, 2.5 µL	6083.0550
Vanquish Fluorescence Detector F with Dual PTM	VF-D51-A
Standard flow cell, biocompatible, 8 µL	6079.4230

*Updated injection valve (6036.2510) and firmware version ≥2.04 required. In case of any questions, please contact customer service.

Sample preparation

A 21-day pack of a drug product labeled to contain 3 mg of drospirenone and 0.02 mg of ethinylestradiol per tablet was purchased from a local pharmacy. Seven tablets were prepared according to the USP dissolution test 2.[1] Each tablet was stirred in 900 mL of water for 30 min. After that, a portion was centrifuged for 15 min and the supernatant was transferred to autosampler vials for analysis.

Standard preparation

A standard solution was prepared according to the USP dissolution test 2.[1] 6 mg of drospirenone were dissolved in acetonitrile and filled up to 10 mL (stock D), 2 mg of ethinylestradiol were dissolved in acetonitrile and filled up to 10 mL, and 1 mL of this solution was diluted 10-fold with acetonitrile (stock EE). Proper volumes of both stock solutions were diluted with water in one 1,000 mL flask to obtain concentrations of 3.333 mg/L D and 0.022 mg/L EE (dissolution standard), which equals the target sample concentration (3 mg D and 0.02 mg EE dissolved in 900 mL water).

For low volume injection tests, the same volumes of stock solutions were diluted with water in one 10 mL flask to obtain the 100-fold concentration (low volume injection standard).

Chromatographic conditions

Table 1. Chromatographic conditions

Column	Thermo Scientific™ Hypersil Gold™ 4.6 x 100 mm, 3 µm (P/N 25003-104630)
Mobile phase	Water/methanol/acetonitrile (55/5/40; v/v/v)
Flow rate	1 mL/min
Run time	11 min
Column temperature	30 °C (forced air, active preheating)
Autosampler temperature	5 °C
Needle wash	20 s, after draw, with mobile phase
Injection volume	200 µL for dissolution testing 2–1,000 µL for repeatability testing
Draw speed	2 µL/s for 2–200 µL injection volumes 10 µL/s for 500–1,000 µL injection volumes
Detector settings	UV: 260 nm, 5 Hz, 1 s response time FLD: excitation 280 nm, emission 310 nm, 5 Hz, lamp mode standard, sensitivity 6 for 2 and 200 µL injections, sensitivity 5 for the other injection volumes

A “PrepareNextInjection” command was inserted in the instrument method script editor at 6 min.

Chromatography Data System

The Thermo Scientific™ Chromeleon™ 7.3.1 Chromatography Data System (CDS) was used for data acquisition and analysis.

Results and discussion

To limit the gradient delay volume contribution of the sampler, the Vanquish Flex sampler is equipped with a 25 µL sample loop in its default configuration and a 100 µL metering device. This injection volume range (0.01–25 µL) covers the majority of common HPLC and UHPLC applications. However, in the case of extended volume requirements, there are several optional loop sizes (100, 250, 1,000 µL) available. The complete injection volume range can be handled by the same precise 100 µL metering device by multiple draw cycles, avoiding the need to change the metering device to a larger volume.

The dissolution test of dropirenone (D) and ethinylestradiol (EE) tablets required an injection volume of 200 µL, so the 250 µL loop was installed. Figure 1 depicts an overlay of a standard and a sample chromatogram, with the D peak visible in the UV trace and the EE peak in the FLD trace. The system suitability criteria of the test required a tailing factor ≤2 and a relative standard deviation in peak area of ≤3% for both peaks in the standard solution, which were easily met. The tailing factor was 0.92 for both peaks and the peak area relative standard deviation (RSD) over seven injections was 1.04% for the D peak and 0.80% for the EE peak. The results for the seven dissolved tablets are summarized in Table 2 and were calculated using the formula:

$$result\ [\%] = \left(\frac{R_S}{R_{Std}}\right) \times \left(\frac{c_{Std}}{L}\right) \times V \times 100$$

R_S = response sample solution
R_{Std} = response standard solution
c_{Std} = concentration standard solution [mg/mL]
L = label claim (mg/tablet)
V = volume medium (900 mL)

The tolerance criteria stated in the USP monograph give limits of ≥80% of the labeled amount of D and ≥85% of the labeled amount of EE dissolved. The sample results were between 80% and 90% for D and between 97% and 102% for EE in all seven tablets, and thus all comply with the requirements of the USP dissolution test 2.

The Vanquish Flex UHPLC system with the 250 µL loop installed was well-suited to perform the test. However, the 1,000 µL loop would be also an interesting option, particularly if other large volume injection applications are to be run with the system. To evaluate the injection performance with either loop size, the dissolution standard was injected also with the 1,000 µL loop at levels of 200, 500 and 1,000 µL. The almost completely aqueous solvent of the dissolution standard enabled the injection of such high volumes without any solvent mismatch effect distorting the peak shapes. In addition a 100-fold concentrated standard was injected with both loops at 2 and 10 µL. As a consequence of increasing injection volumes in this isocratic LC method, the retention times of the compounds increased as calculated from the time required to move the sample plug to the column.

Table 2. Percentage results of the labeled amounts of D and EE dissolved for seven samples (average over three injections)

	D [%]	EE [%]
Sample 1	89.6	101.6
Sample 2	85.1	100.8
Sample 3	84.8	101.9
Sample 4	86.3	101.7
Sample 5	83.3	99.9
Sample 6	80.3	97.4
Sample 7	84.6	101.3

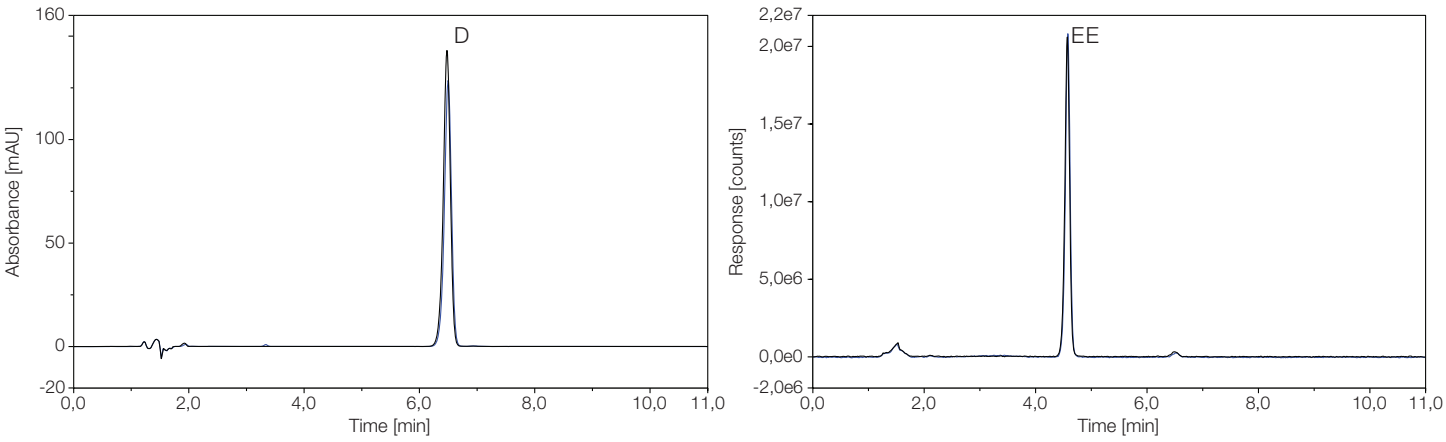

Figure 1. Overlay of chromatograms of the dissolution standard (black) and a dissolved sample (blue). Left: UV for drospirenone; right: FLD for ethinylestradiol

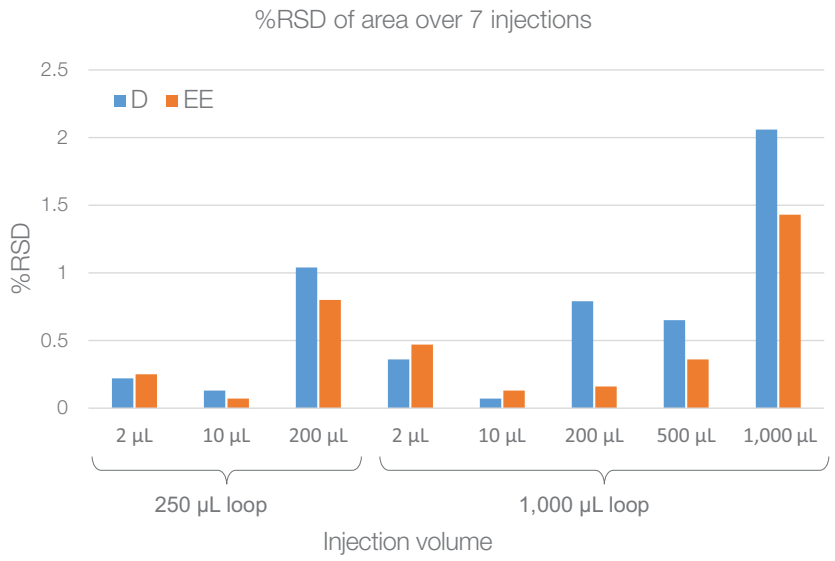

Figure 2. Repeatability of standard injections from 2 to 1,000 µL with the 250 µL and 1,000 µL loop

The outcome of area %RSDs over seven injections is presented in Figure 2. Very good repeatabilty was seen over the complete injection range, with %RSD usually well below 0.5% for 2 and 10 µL injections, which are performed in a single draw. For 200 µL injections %RSD was ≤1% and for 500 and 1000 µL injections it was ≤2%. All injection volumes are managed by the same 100 µL metering device using the multi-draw operation in case of injection volumes above 100 µL. This is beneficial over LC systems that require a swap of the syringe/metering device for larger injection volumes, which usually comes with distinct repeatability decline.

The slightly increased %RSD results for the larger volume injections in Figure 2 may be attributed to the multiple drawing but possibly also to the increased draw speed implemented for the 500 µL and 1,000 µL injections. In large volume injection applications there might be a trade-off between accuracy by slow drawing (particularly at higher viscosity samples) and decreasing the injection cycle time by fast drawing. For the conditions used in the current experiments, the injection preparation took around 5.8 min for 200 µL injections (draw speed 2 µL/s), 9.5 min for 500 µL injections, and 18.5 min for 1000 µL injections (draw speed 10 µL/s). To decrease the effect on the overall run time, a “PrepareNextInjection” command was used in the instrument method at 6 min, causing a switch of the injector valve to bypass to start the injection preparation of the next sample run. With this technique, the latter 5 min of each sample run were utilized in parallel with sample drawing, which for example allowed an injection every 11.8 min instead of every 16.8 min for the 200 µL injections. Readers interested in more details on throughput increase by injection preparation may also refer to Reference 3.

Conclusion

- The Vanquish Flex UHPLC system with 200 µL and 1,000 µL injection loops is well-suited to run dissolution testing applications with injection volumes up to 1,000 µL.
- All tested samples complied with the USP dissolution test tolerances.
- Very good repeatability was shown over the injection range of 2 to 1,000 µL.

References

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