

Fasted State Dissolution Protocol

Introduction

This protocol is a standardized method for running dissolution tests on immediate release oral dosage formulations in fasted media, with HPLC-UV analysis.

The objective is to make sure the dissolution profiles of the test product match the reference drug exactly, thus increasing the likelihood of achieving bioequivalence. Do not worry if the dissolution is not 100% complete, this is precisely why biorelevant media are so useful, as they offer insight and understanding into how drug release may occur *in vivo*.

The 'Pre-experimental' tips section is strongly advised when experimenting on a test product for the first time, to ensure accuracy and reliability of results

Otherwise, skip ahead to the protocol, which is split into three parts; 'Preparation', 'Dissolution Run' and 'Analysis of Results'.



Pre-experimental tips

The following tips are based around the [ICH guidelines Q2\(R1\)](#) on Validation of Analytical Procedure combined with practical experience from our laboratories. Trial runs should be performed according to following checklist:

Checklist

Dissolution run

1. Sinking or floating

2. Coning

HPLC-UV method

1. No interference

2. Filter compatibility

3. Sample stability

4. Calibration standards

Dissolution run

A single USP2 trial run should be performed with your test dosage in chosen fasted media.

USP2 trial run

- Make up 1L of fasted media
- Turn on USP2 Apparatus
- Set water bath temperature to **37.0C ± 0.5°C**
- Add 900 mL of the fasted media to the chosen trial vessel
- Lower paddles and set rotation speed to 75rpm
- Ensure medium is at **37.0C ± 0.5°C**
- Add your test dosage



Pay attention to the following:

1. Does the dosage form float or sink?

If the capsule floats, and the shell does not disintegrate within 5 minutes, then the use of a sinker is advised. Most capsules, however, disintegrate rapidly within 1-2 minutes in which case a sinker should not be used.

2. Does coning occur at the base of the dissolution vessel?

If coning occurs, the speed should be increased from 75 rpm in 25 rpm increments until no coning is observed. Peak vessels may also be used to overcome coning. The same vessel type & paddle speed should then be used for all dissolution tests of the same drug product.

HPLC-UV method

HPLC-UV analysis enables detection of potential chemical instability of the drug and minimizes risk of interference of the media.

For testing the HPLC-UV analysis, originator stock solution is required. A known amount of reference standard drug is dissolved in solvent (e.g. methanol, DMSO) which is then appropriately diluted in mobile phase to give the originator stock solution.

During USP2 sampling in biorelevant dissolution tests, the sample withdrawn from the vessel is diluted 10x (see page 11), thus the HPLC-UV samples consist of 900 μL mobile phase, and 100 μL media containing an unknown amount of dissolved drug.

The following steps should be followed to make sure the analysis is accurate and reliable.

1. No interference

Confirm that the analysis separates the biorelevant media from the drug peak.

How?

Carry out a HPLC-UV run with samples containing;

- 1000 μL mobile phase
- 900 μL mobile phase, 100 μL biorelevant media
- 900 μL originator stock solution, 100 μL biorelevant media

This checks whether the biorelevant media interferes with the chromatogram, in the presence and absence of drug.

2. Filter compatibility

Ensure the drug doesn't adsorb on the filter.

How?

Adsorption is already minimised by passing 2 mL of the dissolution fluid through the filter prior to taking the sample (*see page sampling steps*), to saturate the filter with the drug

However, if it is a low dose drug or you want to test the filter compatibility, compare the drug content of a stock solution sample before and after filtration. The measurements should give the same 'area under curve' (AUC) value.

3. Sample Stability

Make sure the samples remain stable throughout the test period

How?

Take a sample at a given time point (t_0) from the trial vessel and analyse it immediately. Keep this sample at room temperature and analyse it again after the test period has elapsed (t_1). The measurements from t_0 and t_1 should yield the same AUC value. Samples with low stability may need immediate dilution after sampling.

4. Calibration standards

Ensure the calibration standards accurately cover the range of the dissolution profile.

How?

Calculate the maximum possible drug concentration in the dissolution vessels, and account for the 10x dilution performed during sampling;

$$\text{Maximum drug concentration (mg/mL)} = \frac{\text{Test dosage (mg)}}{(900\text{mL of biorelevant media}) \cdot (10)}$$

The maximum possible sample concentration should be taken as 100%, and calibration standards should be made up using the same reference standard stock solution, which range from 1 to 110%. The box below is an example of calibration standards used in our [ibuprofen dissolution case study](#).

Ibuprofen test dosage (mg) – 600

Maximum drug concentration of HPLC samples after dilution (mg/mL) – 0.0667

Thus 0.0667 mg/mL is 100% dissolved. A stock solution of ibuprofen in mobile phase with a concentration of 0.08 mg/mL was used to make up 7 calibration standards according to the table below.

Stock solution (mL)	Mobile phase (mL)	Biorelevant media (mL)	% Max dissolved	Conc. (mg/mL)
0.9000	0.0000	0.1000	108.0	0.072
0.4000	0.5000	0.1000	48.0	0.032
0.2000	0.7000	0.1000	24.0	0.016
0.1000	0.8000	0.1000	12.0	0.008
0.0500	0.8500	0.1000	6.0	0.004
0.0250	0.8750	0.1000	3.0	0.002
0.0125	0.8875	0.1000	1.5	0.001

The calibration standards should then be run, and the calibration graph should be plotted (concentration against AUC). The R² value of the line should be >0.99 to confirm the precision of the preparation and analysis.

Protocol steps

What is needed (for N=6)

For preparation

- 5.5 L of selected fasted media
- 60 Eppendorf tubes (or similar)
- 66 HPLC vials and caps (60 for dissolution sampling and 6 for HPLC calibration standards)
- Calibrated pH meter
- Thermometer
- USP2 apparatus

For the dissolution run

- 6 syringe filters (0.45 µm) Nylon with glass prefilter
- 6 syringes
- 6 units of your test product
- 1 digital timer
- USP2 apparatus (including cannulas)

For the analysis

- HPLC-UV apparatus

Preparation

Step 1

Make Biorelevant Medium

- 5.5 L of media is required
- Preparation instructions at www.biorelevant.com/FFF
- N.B. The media needs to stand for 2 hours



Step 2

Start USP2 Apparatus

- Turn on USP2 Apparatus
- Set water bath temperature to $37.0\text{C} \pm 0.5^{\circ}\text{C}$



Step 3

Add Biorelevant Medium

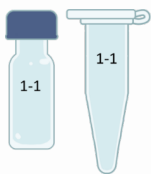
- Add 900 mL of chosen fasted medium to each dissolution vessel
- Lower USP2 paddles and set selected paddle speed, typically at 75 rpm (see 'Pre-experimental tips' for details)
- Ensure medium is at $37.0\text{C} \pm 0.5^{\circ}\text{C}$



Step 4

Labelling

Label all 60 Eppendorf tubes and 60 HPLC vials following the matrix on the right



	Vessel 1	Vessel 2	Vessel 3	Vessel 4	Vessel 5	Vessel 6
Sample 1	1-1	2-1	3-1	4-1	5-1	6-1
Sample 2	1-2	2-2	3-2	4-2	5-2	6-2
Sample 3	1-3	2-3	3-3	4-3	5-3	6-3
Sample 4	1-4	2-4	3-4	4-4	5-4	6-4
Sample 5	1-5	2-5	3-5	4-5	5-5	6-5
Sample 6	1-6	2-6	3-6	4-6	5-6	6-6
Sample 7	1-7	2-7	3-7	4-7	5-7	6-7
Sample 8	1-8	2-8	3-8	4-8	5-8	6-8
Sample 9	1-9	2-9	3-9	4-9	5-9	6-9
Sample 10	1-10	2-10	3-10	4-10	5-10	6-10

Step 5

Prepare Calibration Standards

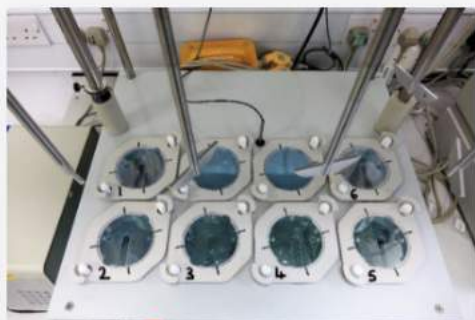
- Make up HPLC mobile phase
- Prepare and label a minimum of 6 standards made from the same Originator Reference stock solution; the concentrations should cover 1% to 110% of the maximum drug concentration (see 'Pre-experimental tips')
- Mix (vortex) the samples until homogenous



Dissolution Run

USP2 set-up

Carry out a HPLC-UV run with samples containing



Step 1

Label the vessels of the USP2 that are to be samples



Step 2

Ensure the cannula is in the slanted position when not sampling, and only in the downward position during sampling



Step 3

Add your first dosage to vessel 1 and mark it as 0 minutes. To prevent damage to the dosage form, use latex or nitrile gloves, forceps or tweezers

USP2 sampling

See sampling schedule below



Step 1

With the cannula in the downward position, withdraw 3mL of dissolution medium into the syringe



Step 2

Detach syringe from cannula and attach syringe filter. Filter 2mL back into the vessel



Step 3

Filter the remaining 1mL into the appropriately labelled Eppendorf tube. Replace the cannula in the slanted position until the next sampling

Preparing HPLC samples



Step 1

Perform a 10x dilution of the dissolution samples with mobile phase into the corresponding autosampler vial, using a pipette (e.g. 100µL of dissolution sample, 900µL of mobile phase).*

Step 2

Vortex samples until thoroughly mixed.

Step 3

Place vials on the HPLC autosampler rack in order of sampling timepoints (1-1, 2-1, 3-1 etc).

* [N.B. 10x dilution is not fixed, smaller dilutions for less soluble drugs may be more appropriate].

Sampling Schedule for 6 dosage units (n =6) for testing one formulation in fasted state biorelevant media

	Vessel 1	Vessel 2	Vessel 3	Vessel 4	Vessel 5	Vessel 6
Addition of tablet/	00:00:00	00:00:50	00:01:40	00:02:30	00:03:20	00:04:10
Sample 1	00:05:00	00:05:50	00:06:40	00:07:30	00:08:20	00:09:10
Sample 2	00:10:00	00:10:50	00:11:40	00:12:30	00:13:20	00:14:10
Sample 3	00:15:00	00:15:50	00:16:40	00:17:30	00:18:20	00:19:10
Sample 4	00:20:00	00:20:50	00:21:40	00:22:30	00:23:20	00:24:10
Sample 5	00:25:00	00:25:50	00:26:40	00:27:30	00:28:20	00:29:10
Sample 6	00:30:00	00:30:50	00:31:40	00:32:30	00:33:20	00:34:10
Sample 7	00:45:00	00:45:50	00:46:40	00:47:30	00:48:20	00:49:10
Start preparing the samples for HPLC in between sampling points						
Sample 8	01:00:00	01:00:50	01:01:40	01:02:30	01:03:20	01:04:10
Sample 9	01:30:00	01:30:50	01:31:40	01:32:30	01:33:20	01:34:10
Sample 10	02:00:00	02:00:50	02:01:40	02:02:30	02:03:20	02:04:10

Run HPLC sequence for the dissolution samples that have been placed on the autosampler rack using an appropriate HPLC-UV method.

Option: record pH in each vessel at the end of dissolution run. pH recording is important for basic and acidic drugs and their salts.

Analysis of Results

HPLC results

- Once you have checked the chromatogram peaks have been integrated correctly by the HPLC software, calculate the concentration of the samples by mapping the area under curve (AUC) values onto the standard calibration graph.
- Plot the mean % drug dissolution of the 6 vessels at each time point vs time (e.g. [ibuprofen case study](#)).
- Also report the standard deviation at each time point. A high SD after 20 minutes could be due to several factors, such as coning, or super-saturation of the drug.
- Results are ready for interpretation

If the release profile of the test product and reference drug do not match, the formulation must be modified accordingly.

In certain instances, dissolution profiles that appear to match *in vitro* still might not be bioequivalent *in vivo*. This may be due to biological reasons, or the biopharmaceutical properties of the drug, such as differences in enzyme metabolism between test subjects.

If you have any questions, contact us on support@biorelevant.com.