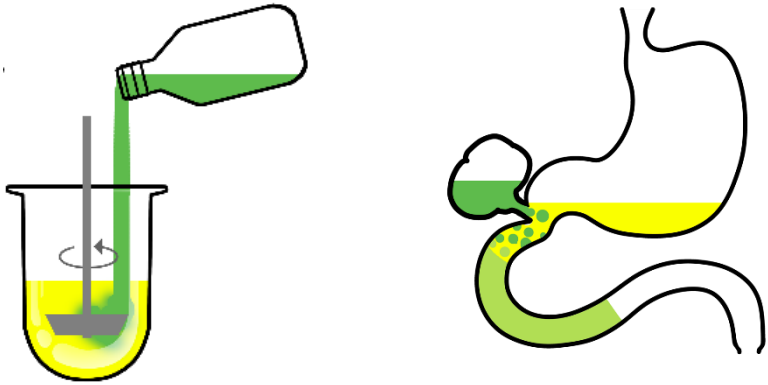


Comparative Two Stage Biorelevant Dissolution Guide

Biorelevant

This guide uses the Two Stage Biorelevant Dissolution Kit

- ✓ Simulate how a drug product behaves when it contacts the stomach fluid (FaSSGF) and then when the fluid is converted into intestinal fluid (FaSSIF)
- ✓ Particularly important for comparing different formulations of basic drugs with a higher solubility at acidic stomach pH compared to higher intestinal pH
- ✓ Results will reveal the drug's tendency to either supersaturate or precipitate in gastrointestinal fluids
- ✓ Results can help formulation development and optimisation

The **Two Stage Biorelevant Dissolution Kit** (Product Code: 2ST-KIT) contains everything you need to run this experiment and it is available to buy here: https://biorelevant.com/Two-Stage-Biorelevant-Dissolution/buy/

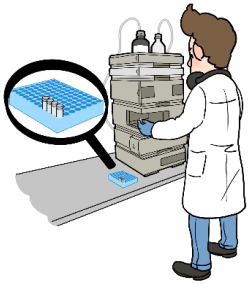
TIP

Before carrying out two stage dissolution, it is strongly suggested to carry out dissolution of Reference and Test formulations in FaSSIF (900mL per vessel). For comparative biorelevant dissolution follow this link https://biorelevant.com/#dissolution_wizard_tab

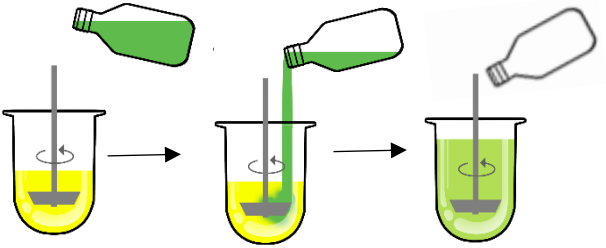
Experiment:	Comparative two stage biorelevant dissolution
Dosage form:	Immediate release (IR) tablet or capsule
Equipment:	USP apparatus 2, HPLC

Objective

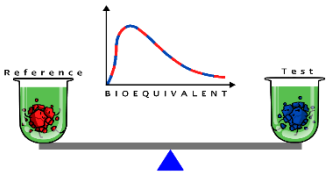
a. Evaluate HPLC method



b. Carry out two stage biorelevant dissolution



c. Compare Test Formulation dissolution profile with Reference



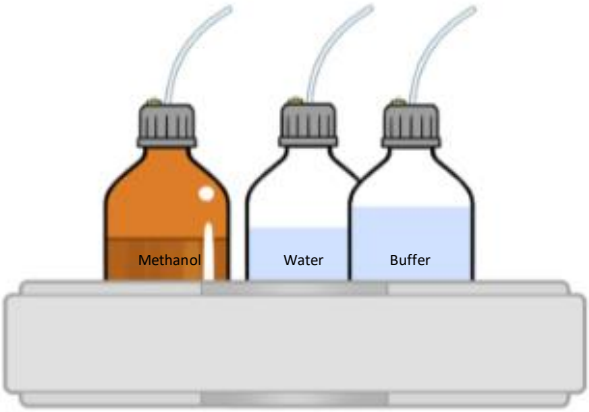
SECTION A: Evaluate HPLC Method

A1) FaSSIF preparation

- ✓ Prepare FaSSIF medium with 3F Powder® and the diluted FaSSIF Buffer Concentrate for HPLC testing using our online Media Prep Tool. Typically, about 300mL of "FaSSIF" are required

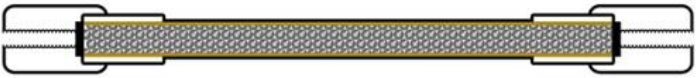
A2) Mobile phase

- ⓘ *A monograph or QC HPLC method can normally be used as a starting point for analysis. Methanol is the preferred organic solvent for mobile phase in combination with appropriate buffer*



A3) Stationary phase

- *Check column suitability for drug analysis*
- *Monograph or QC methods with C18 columns (5µm and 3µm) can typically be used*



A4) Calculate the Maximum theoretical drug concentration

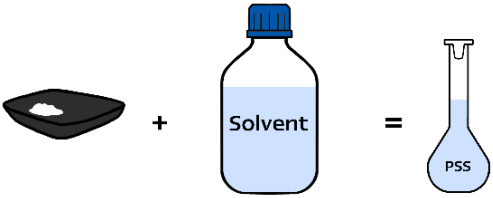
$$\text{Theoretical max drug conc.} = \frac{\text{Dose of drug substance in formulation}}{\text{Volume in the dissolution vessel} *}$$

$*$ recommended volume is 900 mL for FaSSIF

These values will help you establish the amount of drug required to prepare Primary standard solution.

A5) Primary standard solution (PSS)

- *Organic Solvent (e.g. methanol and/or DMSO) of monograph or QC methods can typically be used to dissolve drug substance to prepare PSS*



A6) Diluent for standards and dissolution samples

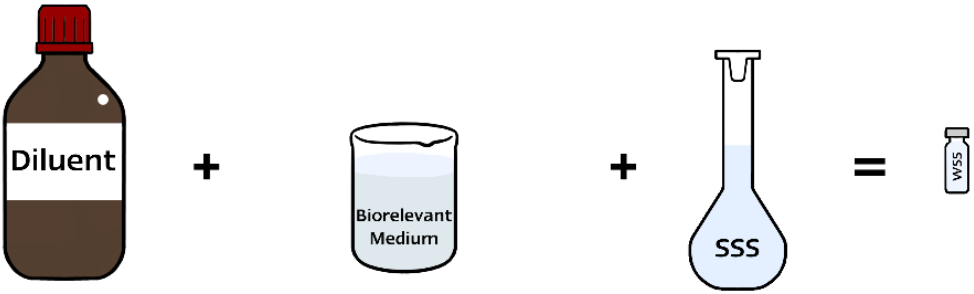
- *The diluent of monograph or QC methods can be used if compatible with FaSSIF*
- *Acetonitrile is **NOT** recommended*
- *Methanol with water (buffered or unbuffered) mixtures can generally be used for dilution of PSS to prepare **secondary standard solution (SSS)** and dilution of **dissolution samples***

ⓘ *Dilute the FaSSIF with diluent, checking different dilution ratios and observe homogeneity and physical stability*

ⓘ *Diluted samples should look homogenous*

ⓘ *Keep the matrix similar;*

- *Use diluent (optionally), FaSSIF and SSS to prepare* ***working standard solution (WSS)***
- *Dilute filtered dissolution samples with diluent*



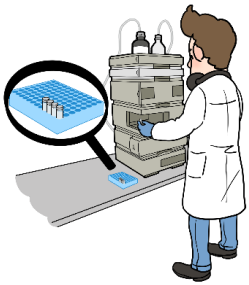
A7) Check interference and stability of fresh samples

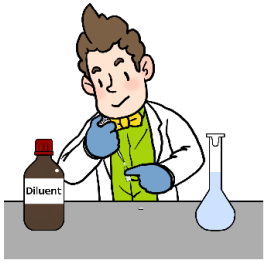
- ✓ Prepare **diluted samples** of:
  - FaSSIF
  - drug without FaSSIF
  - drug with FaSSIF (e.g. WSS)
  - dosage form (e.g. drug and excipients) with FaSSIF

- ✓ Check freshly diluted samples have no precipitate

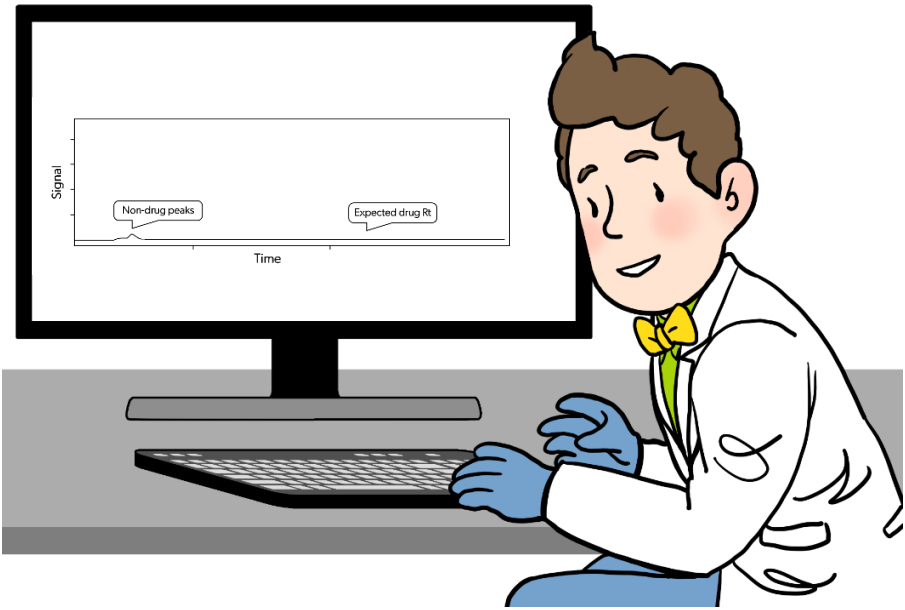
- ✓ Inject samples to check interference and chemical stability
- ✓ Store samples for stability (typically 24hours)

- ✓ Do not inject
- ✓ Adjust diluent and/or ratio to keep samples homogenous





✓ Check non-drug peaks do not interfere

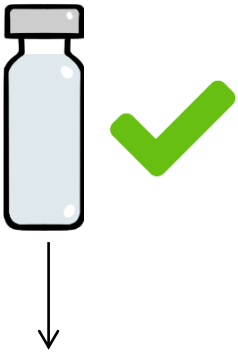


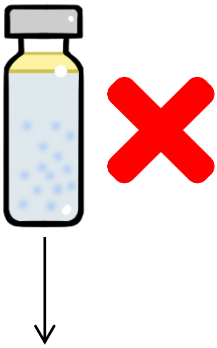
✓ Determine drug peak area and check quality of chromatograms

A8) Stability of stored samples

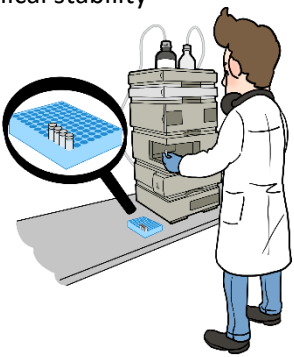
- ✓ Re-inspect stored **diluted samples** which were previously prepared in **Section A7**:
  - FaSSIF
  - drug without FaSSIF
  - drug with FaSSIF (e.g. WSS)
  - dosage form (e.g. drug and excipients) with FaSSIF

- ✓ Re-check stored diluted samples have no precipitate

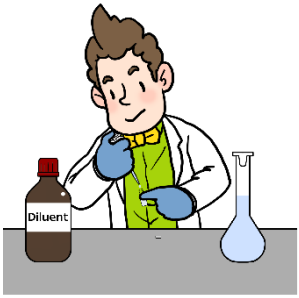




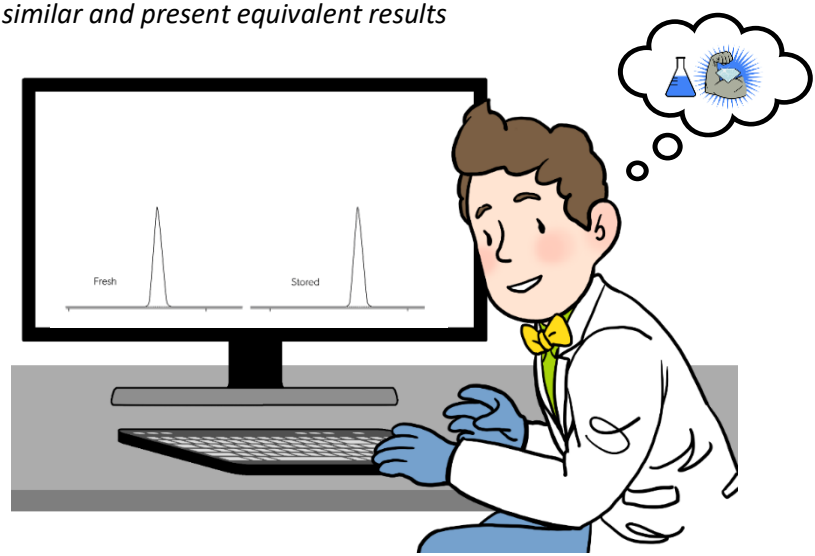
- ✓ Re-inject stored samples to check chemical stability



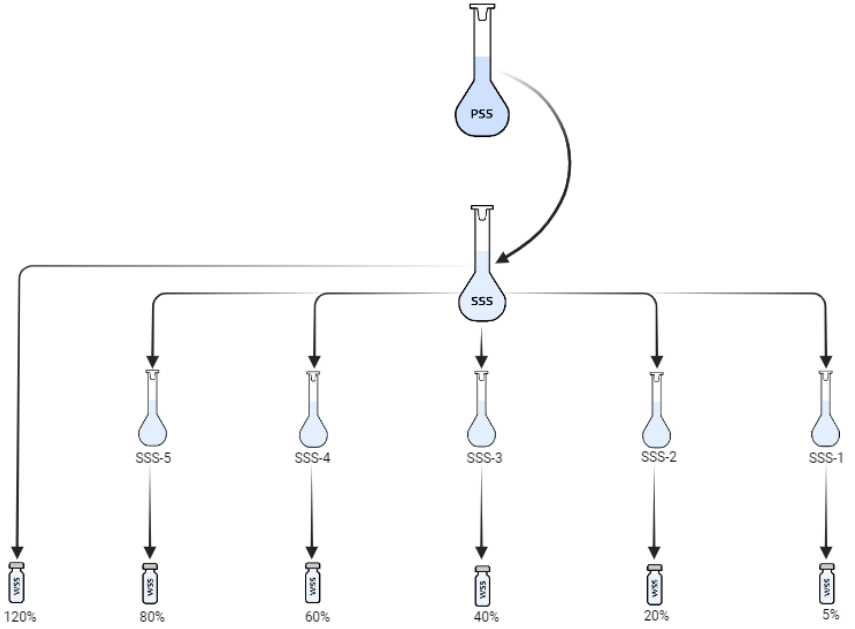
- ✓ Do not re-inject
- ✓ Re-adjust diluent and/or ratio to keep stored samples homogenous



ⓘ *Chromatogram drug peaks of fresh and stored samples should look similar and present equivalent results*



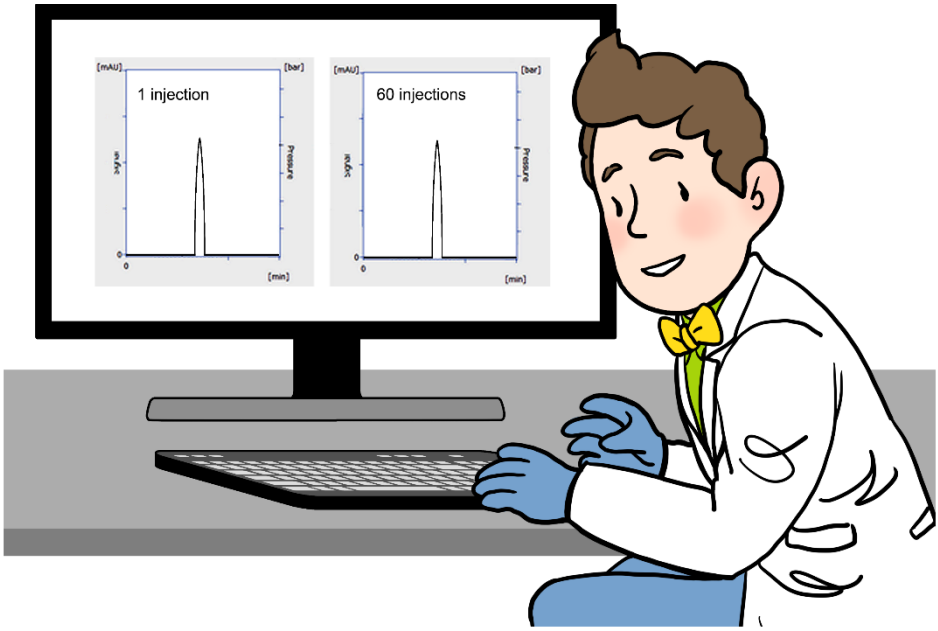
ⓘ *Establish linearity for range of analysis*



ⓘ *Establish limit of (drug) quantification*

A9) Specificity and precision

- ✓ Carry out multiple injections of diluted sample containing dosage form with dissolution medium
- ✓ Check system and chromatographic properties do not substantially change



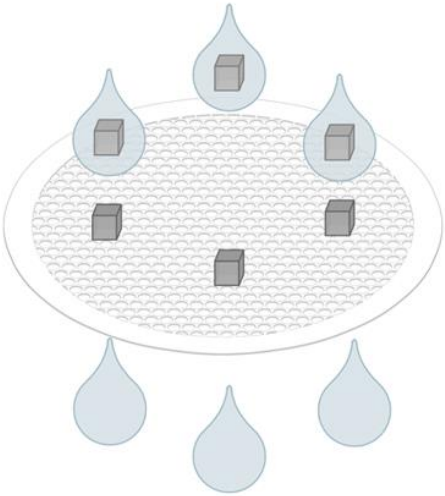
ⓘ *If system properties change, consider washing cycles and/or a guard column*

ⓘ *If chromatographic properties change, consider adjusting method (for example flow rate, temperature, injection volume, solvent ratios)*

ⓘ *If further adaptions are still required, consider modifications (for example changing mobile phase, try a gradient or different solid phase)*

A10) Filter adsorption

- ⓘ *Our 13mm diameter, 0.45µm GMF, inside a 25 mm casing with Luer lock filters are typically recommended for manual sampling. These filters do not leach into biorelevant media. 70* filters are included with the two stage biorelevant dissolution kit
- ⓘ A fresh filter should be used for each sampling time point



- ✓ Check filter does not adsorb drug at a low concentration, for example 10% to 20% of drug release
- ✓ If filter adsorbs, determine volume needed to pre-saturate

SECTION B: DISSOLUTION

B1) Media Preparation for two stage biorelevant dissolution

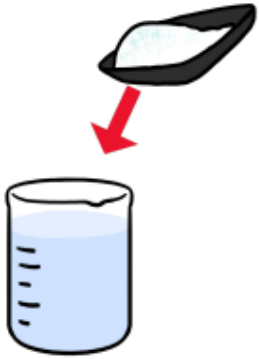
- ⓘ *450mL of FaSSGF and 450mL of Concentrated FaSSIF are required for each vessel*
- ⓘ *The preparation methods for n = 6 vessels are provided below:*

B1a) FaSSGF Preparation *(2.8L includes 100mL overage)*:

1) MAKE BUFFER

-103.0g of FaSSGF Buffer Concentrate
-2693g of purified water

2) ADD POWDER



-Add 0.167g of 3F Powder to the buffer

3) STIR

-Stir until dissolved

4) READY TO USE

-Use medium within 48 hours

B1b) Concentrated FaSSIF *(2.8L includes 100 mL overage 100 mL overage)*:

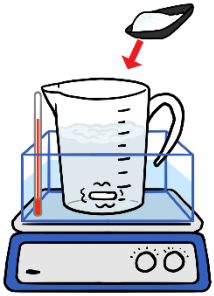
Important: For optimal reproducibility prepare this Concentrated FaSSIF just before starting the dissolution and use within 3 hours of preparation.

1) Make Two Stage FaSSIF Buffer

- **285.7g** of Two Stage Buffer Concentrate
- **2506.8g** of purified water

2) Pre- Heat 37°C

3) Add Powder



- Add **12.38g** 3F Powder® to the Pre-heated Buffer
- Stir until dissolved

4) Weigh Concentrated FaSSIF

- Weigh **452.3g** (450 mL) of "Concentrated FaSSIF" into a container for each dissolution vessel

5) Maintain at 37◦C

B2) Dissolution Parameters

ⓘ *In general, biorelevant dissolution should be performed with the following parameters*

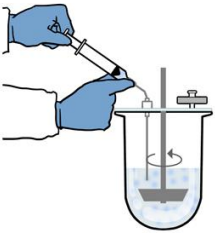
B2a) For FaSSGF

USP apparatus 2

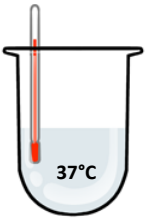
Sinker*

***if capsule floats**

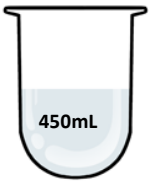
Manual sampling



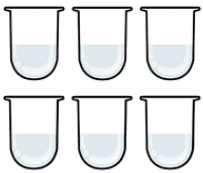
Temperature



Media volume

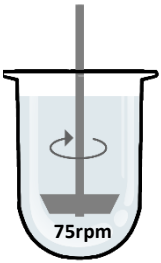


Test n≥3 vs Ref n≥3

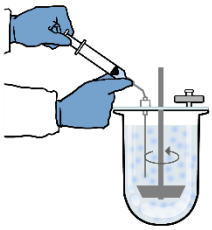


B2b) After addition of Concentrated FaSSIF

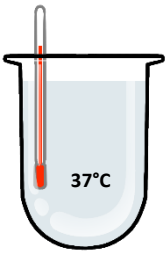
USP apparatus 2



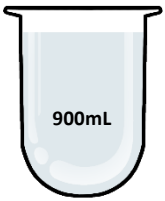
Manual sampling



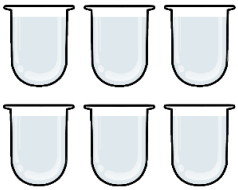
Temperature



Media Volume (after addition)



Test n≥3 vs Ref n≥3



B3) Dissolution Sampling

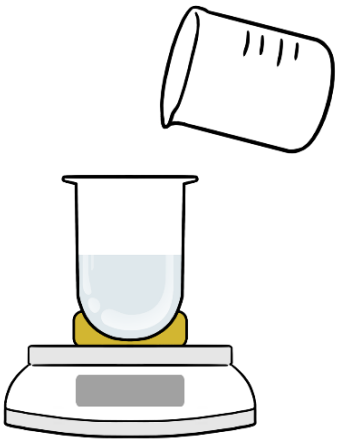
- ⓘ *Perform sampling at:*

Medium	*Sampling time points minutes*
FaSSGF	*5, 10, 20, 30, 45, 60*
After addition of Concentrated FaSSIF	*65, 75, 90, 120, 180*

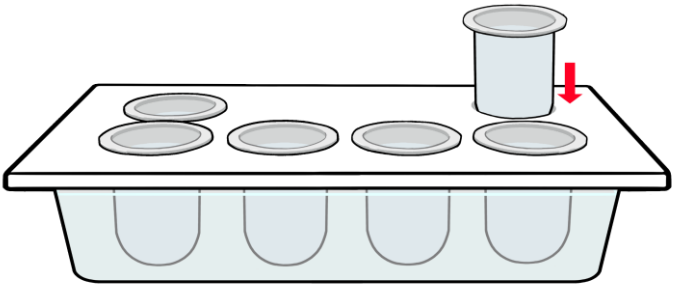
- ⓘ *Carefully observe and note the disintegration behaviour of the immediate release dosage form for the first 2 to 3 minutes*
- ⓘ *After addition of Concentrated FaSSIF to FaSSGF, carefully observe the behaviour of drug within the vessels for least 5 minutes and note if there is a change in appearance*

B4) FaSSGF dissolution set up

- ✓ Fill each vessel with 450mL of FaSSGF

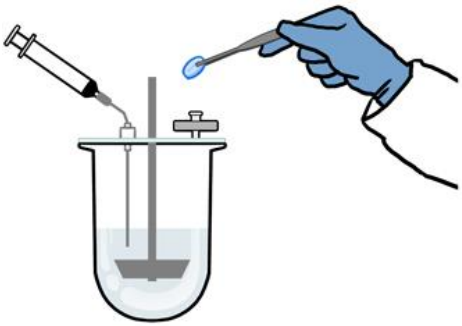


- ✓ Warm to 37°C

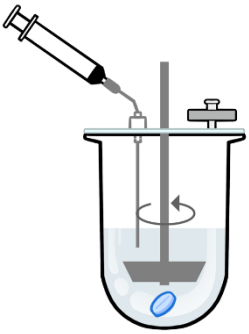


B5) Start FaSSGF dissolution

- ✓ Add formulation

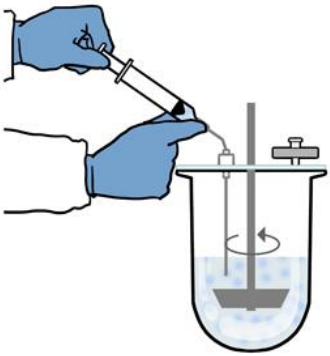


- ✓ Start rotating spindles and timer

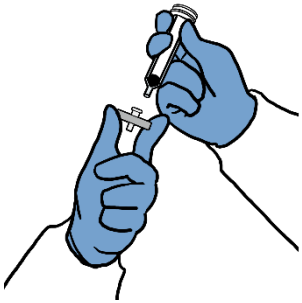


B6) FaSSGF sampling

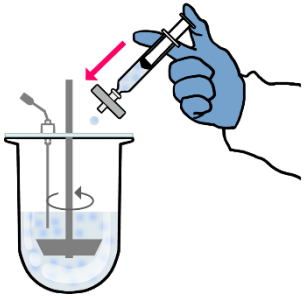
- ✓ Withdraw sample



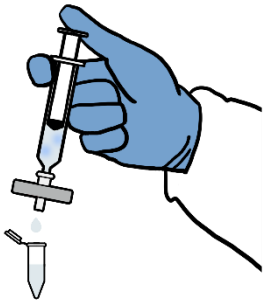
- ✓ Attach fresh filter



- ✓ Pre-saturate filter (see Section A10) and RETURN filtrate back to vessel

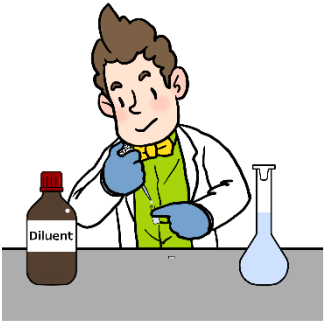


- ✓ Filter and collect remaining dissolution sample (about 1 mL) for analysis



✓ Discard filter, re-attach syringe to cannula

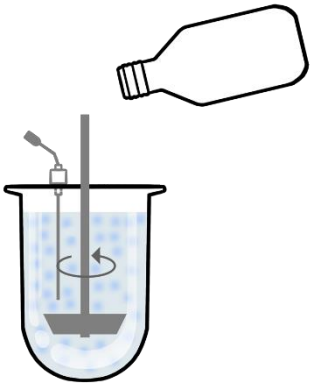
✓ Dilute filtrate immediately (see Section A6)



✓ Repeat sampling at remaining time points until end of FaSSGF dissolution (60 minutes)

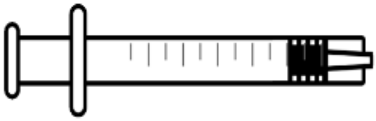
B7) Add Concentrated FaSSIF to FaSSGF

- ✓ At the end of FaSSGF dissolution, pour 450mL of pre-heated **Concentrated FaSSIF** (from B1b) directly into each vessel over 15 seconds

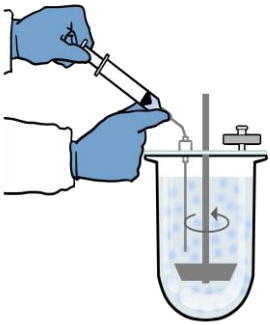


B8) Sampling after addition of Concentrated FaSSIF

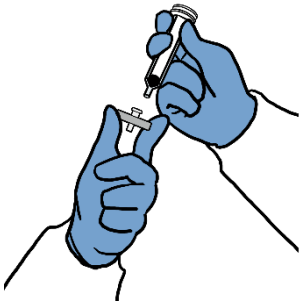
- ✓ Discard the syringe used for FaSSGF Sampling and replace with a new **FRESH Syringe** at the start of the FaSSIF Sampling
- ✓



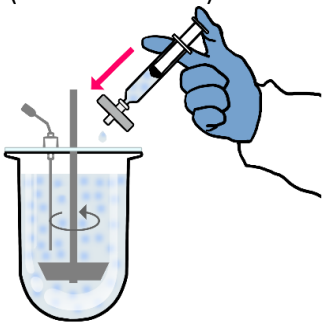
- ✓ 5 minutes after the addition start sampling



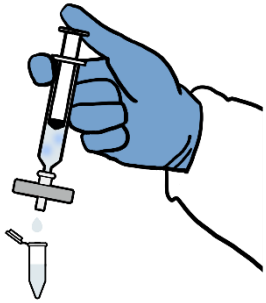
✓ Attach fresh filter



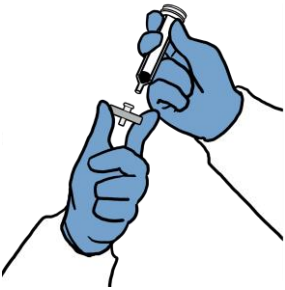
✓ Pre-saturate filter (see Section A10) and return filtrate back to vessel



✓ Filter and collect remaining dissolution sample (about 1mL) for analysis



- ✓ Discard filter, re-attach syringe to cannula

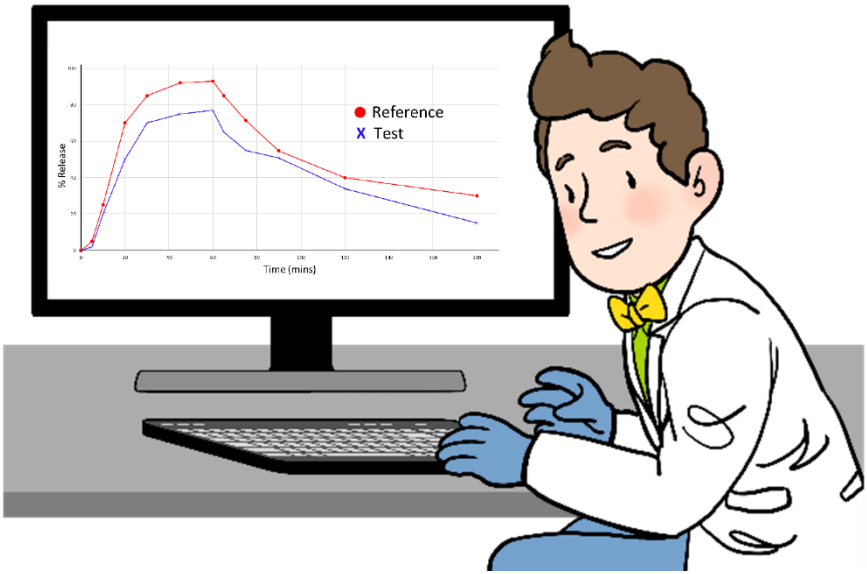


- ✓ Dilute filtrate immediately (see Section A6)

- ✓ Repeat sampling at remaining time points (typically 3hours after start of experiment)

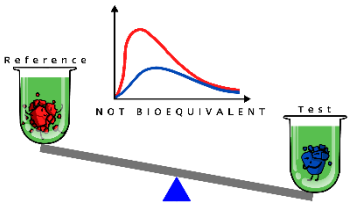
B9) Generate comparative dissolution profiles

- ✓ Analyse samples by HPLC
- ✓ Check the quality of chromatograms
- ✓ Generate dissolution profiles of Test and Reference Drug Products



The goals are:

- ⓘ *Understand dissolution behaviour after conversion of the stomach to intestinal fluid*
- ⓘ *If profile of Test Formulation does not match the Reference, modify Test Formulation so that the dissolution profile matches Reference Product*



ⓘ *For troubleshooting, contact our Help Desk*

ⓘ *Read our Learning Centre posts for more detailed information*