Specialized Laboratory for Drug production (N111049)

Instructions

Solid dosage forms testing:
Dissolution test

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Study program: Drug synthesis and production

Study field: Drug production

Location: S25b
**Introduction**

Ensuring the sufficient bioavailability is the key aspect during the development of a new per oral dosage form. For the solid dosage forms, the bioavailability can be given by the release rate of an active pharmaceutical ingredient (API) from the dosage form. The release rate can be determined in vitro by the dissolution test. The requirements on the dissolution test including the description of the dissolution apparatus, performance and evaluation of the results are stated in the Czech, European and American Pharmacopeia. According to the Czech Pharmacopeia, per oral solid dosage forms are divided, with respect to the dissolution test, into the following groups: solid dosage forms with conventional (unmodified) release of API, solid dosage forms with prolonged release of API and solid dosage forms with retarded release of API. The individual dosage forms differs in the dissolution test performance, when the change of a dissolution medium is or is not necessary, as well as in subsequent evaluation. The first class of the dosage forms mentioned above must fulfill only one requirement, i.e. to release the minimal specified amount of API in the specified period of time. The both remaining dosage forms must fulfill at least two limits or the entire dissolution profile is evaluated.

The aim of this work will be the performance of the dissolution test of tablets containing ibuprofen.

**Tasks**

1. Familiarize yourselves with the chapter Dissolution test of the solid dosage forms in the USP
2. Find the optimal value of the paddle rotation speed based on the visual evaluation of the disintegrated dosage form behavior.
3. Perform the dissolution tests of the given form of ibuprofen in two dissolution media, the first of which represents conditions in a stomach (pH = 1,2) and the second of pH = 7,2 simulates further sections of a gastrointestinal tract.
4. Monitor visually the dissolution test and discuss the differences between tablet disintegration, the appearance of the dissolution medium and bubbles formation.
5. Construct the dissolution profiles (dependence of the API released on time) of all the samples and discuss the differences between both formulations and the influence of the dissolution medium on the API release.

**Instruments and procedures**

**Preparation of the dissolution media and dissolution apparatus:**

The dissolution test will be performed in the dissolution apparatus Sotax AT7-smart with the paddle arrangement (Fig. 1).
Caution! The components of the apparatus including the dissolution vessels may come into contact only with water and dissolution medium. By no means, do not use any organic solvents including ethanol, as this may cause the devaluation of polycarbonate parts.

Use degased demineralized water for the dissolution media preparation. At first, heat the water to approx. 40 °C and then degas in an ultrasonic bath. If this is not possible due to utilization of the laboratory, Carry out the degasing by boiling the water. The volume of the dissolution media is 1000 ml.

**Dissolution medium A**
Prepare the dissolution medium A by dissolving 6.8g KH$_2$PO$_4$ and 0.9 NaOH in the given volume of degased demineralized water. The resulting solution has pH about 7.2 and significant ionic strength and buffer capacity.

**Dissolution medium B**
Prepare the dissolution medium B by adjusting the demineralized water pH value to 1.2 by HCl 36 % (ρ = 1.18 g.cm$^{-3}$) addition. Calculate the necessary amount of water.

1. Place the dissolution vessel into the dissolution apparatus and fix it with three plastic holders
2. Measure the necessary volume of deionized water using the graduated cylinder and put it into the dissolution vessel.
3. Add the necessary amount of HCl, KH$_2$PO$_4$ or NaOH
4. In this manner, place all seven dissolution vessels sequentially.
5. Close the top of the dissolution apparatus carefully to prevent the dissolution vessels and thermometer from damage.
6. Insert the thermometer and switch on the dissolution apparatus, stirrer and thermostat. Then specify the dissolution method.$^1$
7. Put cannulas with PP filter in the holes in the top of the dissolution apparatus and attach syringes for sampling.

$^1$ View the video instruction manual and the dissolution method specification at http://youtu.be/Wr_F2mzLbjl
**Optimal stirrer speed assessment**

1. Using the button on the right side of the dissolution apparatus insert the tablet of APO ibuprofen into the dissolution vessel filled with pure degased water, switch on the stirrer and set the stirrer speed on 150 rpm.
2. Wait until the tablet disintegrates, then turn off the stirrer and let the particles of the disintegrated tablet to fall down on the bottom of the dissolution vessel.
3. Set the stirrer speed to 30 rpm, turn it on and observe the movement of the particles.
4. With the passage of 10 rpm gradually increase the rotation speed until the least value in which all particles are in the movement, i.e. there is not a stationary pile of the particles on the bottom of the vessel.

**The course of the dissolution test**

The dissolution test is highly demanding on the fast and correct performance of the necessary acts. Hence, try the insertion of tablets and the sampling without real tablet insertion.

The dissolution test is performed in several vessels at once. As it is not possible to take sample at the same time it is necessary to work with several seconds lag between the sampling. This can be set in the method under item “Stagger”. In this mode, the sample is inserted only to the first vessel in the beginning of the experiment. To the next vessels, the sample is inserted after the lag time. The apparatus shows instructions to the next action and counts down the time to its execution.

1. Place the tablet in advance to the colorful dosing hole and insert it in the specified time by pressing the button on the right side of the apparatus top.
2. Take the samples in 2, 5, 10, 15, 30 and 45 minute using PP syringes.
3. Find the ibuprofen concentration using the UV spectrophotometry.

**Preparation of calibration solutions**

Prepare 4 calibration solutions meeting the entire interval of the measured concentration. For this purpose, calculate the maximal theoretical concentration of ibuprofen, which can arise during the dissolution test. Increase this value by 100mg/l and split the resulting interval to the for equal parts. The concentration of calibration solution will be then as follows:

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\begin{align*}
    c_1 &= c_{\text{max}} + 100 \text{ mg/l} \\
    c_2 &= c_1 \cdot 3/4 \text{ mg/l} \\
    c_3 &= c_1 \cdot 1/2 \text{ mg/l} \\
    c_4 &= c_1 \cdot 1/4 \text{ mg/l}
\end{align*}
\]

Preparation of calibration solutions:

1. Use the dissolution medium A as a solvent.
2. Weight the required amount of ibuprofen analytical standard in the volumetric flask of 50 ml, fill 2/3 of its volume by the solvent, mix the solution with rotational movements and put it to the sonication bath.
3. Fill the flask with the solvent to the mark, close it and mix the solution.
4. Verify that the API is completely dissolved.
Assessment of the API concentration in the samples

Determine the amount of ibuprofen in the sample using UV spectrophotometer Shimadzu UV mini 1240. (Fig. 2)

The initializing of the device takes several minutes so make ahead. Before starting the measurement, it is necessary to find the analytical conditions and establish the calibration relationship between absorbance and concentration. Analyze the samples in the quartz cuvettes. After the opening of the spectrophotometer lid, place the cuvettes to the sample space in the left part of the apparatus. Facing the device, the beam passes from right to left and therefore the transparent windows of cuvettes must be oriented in this direction. It is necessary to close the lid of the device before performing a measurement. Rinse the cuvette at least twice with the tested sample and fill it ca. 0.5 cm below the top.

1. Place the cuvette filled with demineralized water into the sample space before turning on the spectrophotometer. Clean the cuvette and its windows with cellucotton properly, if there is moisture or dirt on its surface.
2. Turn on the device using the power switch on the back side and wait for initializing the device until a control menu appears on the display.

3. Press „2“ on the keyboard of spectrophotometer for choosing „Spectrum.“ Then again press 2“ for a choice „λ range“ and enter the range from 400 to 250 nm. Confirm the values by pressing ENTER.
4. Take the cuvette filled with the calibration solution of the highest concentration and place it in the sample space.

5. Measure the UV spectrum of the solution by pressing START.

6. Find maximum on the wave spectrum. If the value of absorbance in this maximum is lower than 2, then you can use this wavelength for subsequent measurement. If the value is higher or it is not possible to identify it, than the solution is too concentrated (Lambert-Beer law is not valid) and it necessary to perform the measurement outside the maximum. Hence, choose a suitable wavelength so that the absorbance value is between 1 – 1,2 and perform the subsequent measurement and this value.

7. Press „RETURN“ repeatedly on the spectrophotometer keyboard to return to main menu. Delete the spectrum if asked by pressing „OK“.

8. Press „1“ on the keyboard for choice „Photometric“.
9. Press „Go to WL“ and enter the chosen wavelength for the measurement. The value of this wavelength should appear in the of the display.

10. Put the cuvette with deionized water in the sample space.

11. Press „Autozero“ on the keyboard. Absorbance indicated in the bottom part of the display should set to zero.

12. Put the cuvette with the sample to the sample space and close the lid. The value of absorbance is displayed immediately on the screen.

13. At first, carry out a blind experiment, i.e. measure the concentration of the pure solvent without an analyte. Then measure the calibration solutions and construct a calibration curve (dependence of concentration on absorbance) with five data points from the absorbance values of the calibration solutions and the absorbance of a blind experiment.

14. Fit the straight line to the calibration curve, assess its linearity and express the calibration equation in the following form: \( c = k_1 \cdot A + k_2 \).

15. Consequently, measure the absorbance of the samples taken during the dissolution test and, using the calibration equation, determine the concentration of ibuprofen in the samples. Then plot the concentration in the chart as a time dependent.

**Protocol requirements**

- Header, briefly stated the aims of the work, procedures of the work
- Calibration dependence, calibration equation, linearity assessment
- Description of the experiment visual observation
- Dissolution profile of the individual tablets or the average dissolution profiles of the formulations. Discussion of the repeatability of the dissolution profile determination according to the chosen statistical measure of variability.
- Discussion of pH influence on the API release
- Discussion of the differences in the API release from both samples based on the dissolution profiles and visual observation.
- Adjust the graphical form of the protocol according to the manual for the protocol of specialized laboratory preparation