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**King Saud University**

**College of Pharmacy**

**Department of Pharmaceutics**

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**Biopharmaceutics**

**PHT 414**

**Laboratory Assignments**

**2014 G – 1435 H**

**Department of Pharmaceutics Biopharmaceutics PHT -414**

**Laboratory Assignments**

**Main subjects which will be covered in the labs.:**

1. You will be know the **pH Partition Theory**, , you will be able to calculate and determine the partition coefficient.

2. You will know the **Effect of pH on the partition coefficient** of acidic and basic drugs

3. Also you will know what **Drug dissolution**, you will also study the Effect of pH on the drug dissolution.

4. Also you will study the Effect of particle size on the **Drug dissolution**.

5. Also you will study the Effect of viscosity on the **Drug dissolution**.

6. Also you will study the Effect of formulation factor on the **Drug dissolution**.

7. Then you will be able to study the **Evaluation of gastrointestinal absorption**, taking in consideration the Workshop on the in vitro, in situ and in vivo methods including selection of animal models.

8. After that, You will continue the study the **Evaluation of gastrointestinal absorption**, the Workshop on the in vitro, in situ and in vivo methods including selection of animal models.

9. Then you will be able to study the **Bioavailability and bioequivalence**, taking in consideration the Workshop on bioavailability, bioequivalence and biowaiver.

10. Then studying the **Evaluation of non-oral drug delivery**, first week the Evaluation of transdermal delivery

11. Then studying the **Evaluation of non-oral drug delivery**, second week the Evaluation of parenteral and ocular delivery.

12. Then studying the **Evaluation of non-oral drug delivery**, second week the Evaluation of Evaluation of inhalation and rectal delivery.

**To attain these specific objectives the following rules must be adhered to in the lab:**

1. All students must wear clean, white lab coats while in the laboratory.

2. All students must follow the instructions of their Teaching Assistant (TA).

3. All students must complete all laboratory assignments. If a lab is missed, the reason for the absence must be discussed with the instructor and a makeup lab arranged. A substantial grade reduction will be given for any assignment which is not completed.

4. No aid is to be given or received **between students** in the lab. Students who exhibit dishonest or unprofessional behavior in the lab may, at the discretion of the instructor receive a failing grade for the laboratory portion of the course.

5. Only a student’s best effort is to be submitted for grading. Receipt of a carelessly prepared Rx will result in a failing grade for the exercise.

6. A name tag with your full name, Pharmacy Student as a title and "I 'm proud to be a Pharmacist" as a badge is a must for every student.

7. Always bring with you : Scientific calculator with Linear regression and graph paper.

**What are the responsibilities of each students:**

1. The attendance of each lab. is a must (from8- 11 a.m and 1-4 p.m.)

2. There will be a quiz/lab. "Practical and theoretical" for the first **6** labs.

3. Then a second quiz/lab. "Theoretical" for the other last 6 labs involving:

a. Subjects covered in labs.

b. Other assignments (paper on one of the lab subjects, Summery report of the work.



**Department of Pharmaceutics Biopharmaceutics PHT -414**

**Introduction to the Course Outline**

**Biopharmaceutics PHT-414**

**Lecturer:** Bushra Al-Quadeib

**Lectures:** One semester - Three hours per week

**Laboratory:** One semester - One (three hour) session per week

**Grading**

|  |  |
| --- | --- |
| Quizzes  Theoretical  Performance (lab.)  Final lab exam. | **3**  **5**  **2**  **10** |
| Total | **20** |

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## Coursework

**LABORATORY PROJECTS’ OUTLINE (414 PHT)**

|  |  |  |
| --- | --- | --- |
| **Week** | **Topic** | **Description** |
| 1 | Introduction | Introduction |
| 2 | pH partition theory | Determination of partition coefficient |
| 3 | Cont. | Effect of pH on the partition coefficient of acidic and basic drugs |
| 4 | Drug dissolution | Effect of pH |
| 5 | Cont. | Effect of particle size |
| 6 | Cont. | Effect of viscosity |
| 7 | Cont. | Effect of formulation factor |
| 8 | Evaluation of gastrointestinal absorption | Workshop on the in vitro, in situ and in vivo methods including selection of animal models |
| 9 | Cont. | Workshop on the in vitro, in situ and in vivo methods including selection of animal models (Continue) |
| 10 | Bioavailability and bioequivalence | Workshop on bioavailability, bioequivalence and biowaiver |
| 11 | Evaluation of non-oral drug delivery | Evaluation of transdermal delivery |
| 12 | Cont. | Evaluation of parenteral and ocular delivery |
| 13 | Cont. | Evaluation of inhalation and rectal delivery |
| 14 | Exam |  |

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**Department of Pharmaceutics Biopharmaceutics PHT -414**

**Lab # 1**

**Partition Coefficient**

**Practical-1:**

**Aim**

Determination of the Partition Coefficient of Citric Acid **(drug)** between Water (**aqueous phase**) and 2-Methylpropan-1-ol (isobutyl alcohol) (**non aqueous phase)**.

**Introduction**

If a solute is added to two immiscible solvents, A and B. in contact with each other, the solute distributes itself between the two and equilibrium is set up between the solute molecules in solvent A and the solute molecules in solvent B. The ratio of the concentration of the solute in the two solvents is



where K is known as the **partition coefficient** or **distribution coefficient**.

**Chemicals**

2-Methylpropan-1-ol (density = 0.805 gdm-3), 0.2 M Citric acid (drug already dissolved in the aqueous phase – water), 0.1 M NaOH, phenolphthalein indicator

**Apparatus**

100 ml separating funnel, titration apparatus, 5 ml pipette

**Procedure**

1. Record the room temperature.

2. Using suitable apparatus pour **25 ml** of the 0.2 M Citric acid solution and **25 ml** of 2-methylpropan-1-ol into a 100 cm3 separating funnel. Stopper the funnel and shake vigorously for 6 minutes. (Release pressure in the funnel by occasionally opening the tap.)

3. Separate approximately **15 ml** of **EACH** layer and collect them in **TWO** clean beakers. (Discard the fraction near the junction of the two layers.)

4. Pipette **5 ml** of the aqueous layer into a titration flask and titrate it with **0.1 M sodium hydroxide solution** using 2 drops of **phenolphthalein indicator**.

5. Using another pipette, deliver **5 ml** of the non-aqueous layer into a titration flask and titrate it with **0.1 M sodium hydroxide solution** using 2 drops of **phenolphthalein indicator**.

7. For each experiment, calculate the ratio of the concentration of citric acid in the non-aqueous layer in relation to the aqueous layer.



**Comment on your results.**

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**Department of Pharmaceutics Biopharmaceutics PHT -414**

**Lab # 2**

**Effect of pH on the Partition Coefficient of acidic and basic drugs**

**Aim**

Determination of the effect of pH on Partition Coefficient of Citric Acid (drug) between **(aqueous phase)** and 2-Methylpropan-1-ol (isobutyl alcohol) **(non aqueous phase).**

**Introduction (pH - Partition Theory)**

For a drug to cross a membrane barrier it must normally be soluble in the lipid material of the membrane to get into membrane it has to be soluble in the aqueous phase as well to get out of the membrane. Many drugs have polar and non-polar characteristics or are weak acids or bases. For drugs which are weak acids or bases the pKa of the drug, the pH of the GI tract fluid and the pH of the blood stream will control the solubility of the drug and thereby the rate of absorption through the membranes lining the GI tract.

Brodie et al. stated that when a drug is ionized it will not be able to get through the lipid membrane, but only when it is non-ionized and therefore has higher lipid solubility.

**Chemicals**

2-Methylpropan-1-ol (density = 0.805 gdm-3), 0.2 M Citric acid (drug already dissolved in the aqueous phase – Buffer solutions with different pH ), 0.1 M NaOH, phenolphthalein indicator

**Apparatus**

100 ml separating funnel, titration apparatus, 5 ml pipette

**Procedure**

1. Record the room temperature.
2. Using suitable apparatus pour 25 ml of the 0.2 M Citric acid solution and 25 ml of 2-methylpropan-1-ol into a 100 cm3 separating funnel. Stopper the funnel and shake vigorously for 6 minutes. (Release pressure in the funnel by occasionally opening the tap.)
3. Separate approximately 15 ml of EACH layer and collect them in TWO clean beakers. (Discard the fraction near the junction of the two layers.)
4. Pipette 5 ml of the aqueous layer into a titration flask and titrate it with 0.1 M sodium hydroxide solution using 2 drops of phenolphthalein indicator.
5. Using another pipette, deliver 5 ml of the non-aqueous layer into a titration flask and titrate it with 0.1 M sodium hydroxide solution using 2 drops of phenolphthalein indicator.
6. Repeat steps (2) to (5) with another separating funnel using either one of the following as aqueous solutions
   1. Acetate buffer pH 4.5
   2. Phosphate buffer Ph 7.2
7. For each experiment, calculate the ratio of the concentration of citric acid in the non-aqueous layer in relation to the aqueous layer.



**Results**

|  |  |  |  |
| --- | --- | --- | --- |
| **Buffer (pH)** | **Volume of 0.1 M NaOH titre for aqueous layer/ cm3** | **Volume of 0.1 M NaOH titre for non-aqueous layer/ cm3** | **Partition coefficient (K)** |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |

**Comment on your results.**

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**Lab # (3-6)**

**Drug Dissolution**

## Drug dissolution

The dissolution test: is an in-vitro test for measuring the time required for a given percentage of the drug substance in a tablet to go into solution under a specified set of conditions.

USP / NF have provided procedures for dissolution testing:

* Apparatus I: in general, a single tablet is placed in a small wire mesh basket fastened to the bottom of the shaft connected to a variable speed motor. The basket is immersed in the dissolution medium contained in 1000 ml flask. The flask is maintained at 37 ± 0.5 ˚C by a constant temperature water bath. Samples of the fluid are withdrawn at specified time intervals to determine the amount of the drug in solution.
* Apparatus II: the same equipment as in apparatus I is used, except that the basket is replaced by a paddle, formed from a blade and a shaft, as the stirring element. The dosage form is allowed to sink to the bottom of the flask before stirring.

Description of a dissolution test in USP/NF monograph specifies:

* + The dissolution test medium, and volume,
  + Which apparatus is to be used,
  + The speed at which the test is to be performed (rpm),
  + The time limit for the test, and
  + The assay procedure.

The test tolerance is expressed as:

* + - The percentage of the labeled amount of the drug dissolved in the time limit.
    - The results are plotted as concentration vs. time. Values for t 50%, t90%, and the percentage dissolved in 30 minutes are used as guides. The value for t50% is the length of time required for 50% of the drug to go into solution.
    - N.B., a common dissolution tolerance in the USP/NF is not less than 75% dissolved in 45 minutes.



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**Lab # 3**

**Effect of Particle size on the Drug Dissolution**

**DISSOLUTION TEST FOR aspirin TABLETS with different particle size (U.S.P. 1995)**

***Conditions:***

* + Apparatus : I (basket)
  + Medium : 500 ml of 0.05 M acetate buffer pH 4.5
  + Temp. : 37 ± 0.5 oC
  + Speed : 50 rpm
  + Time : 45 min.

***Procedure:***

1. Place one tablet in the basket, immerse in the vessel, and then start the apparatus at the above conditions.
2. At specified time intervals (5, 10, 15, 20, 25, 30 and 45 min) withdraw **1 ml** sample from the dissolution medium, through a Millipore filtration unit (polyethylene tube with a cotton), and place the sample in a test tube.
3. Replace the withdrawn sample with **1 ml** fresh acetate buffer kept at 37 ± 0.5o C.
4. Dilute **1 ml** of the collected sample to 5, 10, 20 or 25 ml (dilution factor = 1:5, 1:10, 1:20 or 1:25) with fresh acetate buffer (in a volumetric flask), mix well. ***(Dilution is made if necessary.)***
5. Read the absorbance for the diluted samples at **265 nm** against a blank of acetate buffer.
6. Calculate the concentration of aspirin released (taking **0.036** as the value of **E1mg%**), and express this conc. As a percentage of the labeled amount.
7. Plot the dissolution curve of aspirin (% released vs. time).
8. From the dissolution curve, determine the time required for 80% of the labeled amount of the drug to be released (go into solution), i.e., t 80%.

**USP stated that:** the tablet should release not less than 70% of its content within 30 minutes and not less than 90% of its content within 45 minutes.

**Results of the dissolution of aspirin tablets: (Bayer tablets, 300 mg)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time  (min) | Abs.  at 265 nm | Dilution factor =  ( total vol / vol taken from the sample) | Conc. = x dil factor x 5  (mg/500ml) | % released =x 100 |
| 5 |  |  |  |  |
| 10 |  |  |  |  |
| 15 |  |  |  |  |
| 20 |  |  |  |  |
| 25 |  |  |  |  |
| 30 |  |  |  |  |
| 45 |  |  |  |  |

**Results of the dissolution of aspirin Micronized: ( )**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time  (min) | Abs.  at 265 nm | Dilution factor =  ( total vol / vol taken from the sample) | Conc. = x dil factor x 5  (mg/500ml) | % released =x 100 |
| 5 |  |  |  |  |
| 10 |  |  |  |  |
| 15 |  |  |  |  |
| 20 |  |  |  |  |
| 25 |  |  |  |  |
| 30 |  |  |  |  |
| 45 |  |  |  |  |

* **Plot the Time versus % release on graph paper and comment on the effect of the particle size on the drug released rate??**
* **Determine the t80% of the drug??**
* **Comment on the release of the drug from the products at 30 minute according to USP requirements**



**Department of Pharmaceutics Biopharmaceutics PHT -414**

**Lab # 4**

**Effect of pH on the Drug Dissolution**

**DISSOLUTION TEST FOR aspirin TABLETS (U.S.P. 1995)**

***Conditions:***

* + Apparatus : I (basket)
  + Medium : 500 ml of 0.05 M acetate buffer pH 4.5 ((Officially)
  + Medium : 500 ml of 0.05 M acetate buffer pH 6 ((Unofficially)
  + Medium : 500 ml of 0.05 M acetate buffer pH 3 ((Unofficially)
  + Temp. : 37 ± 0.5 oC
  + Speed : 50 rpm
  + Time : 30 min.

***Procedure:***

1. Place one tablet in the basket, immerse in the vessel, and then start the apparatus at the above conditions.
2. At specified time intervals (5, 10, 15, 20, 25, and 30 min) withdraw **1 ml** sample from the dissolution medium, through a Millipore filtration unit (polyethylene tube with a cotton), and place the sample in a test tube.
3. Replace the withdrawn sample with **1 ml** fresh acetate buffer kept at 37 ± 0.5oC.
4. Dilute 1 ml of the collected sample to 5, 10, 20 or 25 ml (dilution factor = 1:5, 1:10, 1:20 or 1:25) with fresh acetate buffer (in a volumetric flask), mix well. ***(Dilution is made if necessary.)***
5. Read the absorbance for the diluted samples at **265 nm** against a blank of acetate buffer.
6. Calculate the concentration of aspirin released (taking **0.036** as the value of **E1mg%**), and express this conc. As a percentage of the labeled amount.
7. Plot the dissolution curve of aspirin (% released vs. time).
8. From the dissolution curve, determine the time required for 80% of the labeled amount of the drug to be released (go into solution), i.e., t 80%.

**USP stated that:** the tablet should release not less than 70% of its content within 30 minutes. (Officially)

**Results of the dissolution of aspirin tablets: (Bayer tablets, 300 mg) at pH 4.5 (official)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time  (min) | Abs.  at 265 nm | Dilution factor =  ( total vol / vol taken from the sample) | Conc. = x dil factor x 5  (mg/500ml) | % released =x 100 |
| 5 |  |  |  |  |
| 10 |  |  |  |  |
| 15 |  |  |  |  |
| 20 |  |  |  |  |
| 25 |  |  |  |  |
| 30 |  |  |  |  |

**Results of the dissolution of same aspirin tablet at pH 6 (Unofficial)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time  (min) | Abs.  at 265 nm | Dilution factor =  ( total vol / vol taken from the sample) | Conc. = x dil factor x 5  (mg/500ml) | % released =x 100 |
| 5 |  |  |  |  |
| 10 |  |  |  |  |
| 15 |  |  |  |  |
| 20 |  |  |  |  |
| 25 |  |  |  |  |
| 30 |  |  |  |  |

**Results of the dissolution of same aspirin tablet at pH 3 (Unofficial)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time  (min) | Abs.  at 265 nm | Dilution factor =  ( total vol / vol taken from the sample) | Conc. = x dil factor x 5  (mg/500ml) | % released =x 100 |
| 5 |  |  |  |  |
| 10 |  |  |  |  |
| 15 |  |  |  |  |
| 20 |  |  |  |  |
| 25 |  |  |  |  |
| 30 |  |  |  |  |

* **Plot the Time versus % release on graph paper and comment on the effect of the pH on the drug released rate??**
* **Determine the t80% of the drug in different pH media??**
* **Comment on the release of the drug from the product at 30 minute according to USP requirements** (For official medium pH 4.5)



**Department of Pharmaceutics Biopharmaceutics PHT -414**

**Lab # 5**

**Effect of the Viscosity on the Drug Dissolution**

**DISSOLUTION TEST FOR PARACETAMOL TABLETS**

**Tylenol (U.S.P. 1995)**

***Conditions:***

* + Apparatus : II (paddle)
  + Medium : 900 ml of phosphate buffer pH 5.8 in water ((official)
  + Medium : 900 ml of phosphate buffer pH 5.8 in methylcellulose

((Unofficial)

* + Medium : 900 ml of phosphate buffer pH 5.8 simple syrup

((Unofficial)

* + Temp. : 37 ± 0.5 oC
  + Speed : 50 rpm
  + Time : 30 min.

***Procedure:***

1. Place one tablet in the vessel, and then start the apparatus at the above conditions.
2. At specified time intervals (5, 10, 15, 20, 25, 30, and 45 min) withdraw **1 ml** sample through a Millipore filtration unit (polyethylene tube with a cotton), and place the sample in a test tube. Replace the withdrawn sample with **1 ml** fresh phosphate buffer kept at 37 ± 0.5 o C.
3. Dilute **1 ml** of the collected sample to 20 or 25 ml (dilution factor = 1:20 or 1:25) with fresh phosphate buffer (in a volumetric flask) , mix well.
4. Read the absorbance for the diluted samples at 243 nm against a blank of phosphate buffer.
5. Calculate the concentration of paracetamol released (from the LR equation of the line obtained by plotting the standard calibration curve) and express this conc. As a percentage of the labeled amount.
6. Plot the dissolution curve of paracetamol (% released vs. time).
7. From the dissolution curve, determine the time required for 80% of the labeled amount of the drug to be released (go into solution), i.e., t 80%.

**USP stated that:** the tablet should release not less than 80% of its content within 30 minutes and not less than 90% of its content within 45 minutes.

**Results of the dissolution of paracetamol tablets Official Test:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time  (min) | Abs.  at 243 nm | Dilution factor =  ( total vol / vol taken from the sample) | Conc. =[(abs-a)/b]x dil factor x 900 /1000  (mg/900ml) | % released = (conc./original strength) x 100 |
| 5 |  |  |  |  |
| 10 |  |  |  |  |
| 15 |  |  |  |  |
| 20 |  |  |  |  |
| 25 |  |  |  |  |
| 30 |  |  |  |  |

**Results of the dissolution of paracetamol tablets Unufficial Test (Methylcellulose):**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time  (min) | Abs.  at 243 nm | Dilution factor =  ( total vol / vol taken from the sample) | Conc. =[(abs-a)/b]x dil factor x 900 /1000  (mg/900ml) | % released = (conc./original strength) x 100 |
| 5 |  |  |  |  |
| 10 |  |  |  |  |
| 15 |  |  |  |  |
| 20 |  |  |  |  |
| 25 |  |  |  |  |
| 30 |  |  |  |  |

**Results of the dissolution of paracetamol tablets Unofficial Test (Simple syrup):**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time  (min) | Abs.  at 243 nm | Dilution factor =  ( total vol / vol taken from the sample) | Conc. =[(abs-a)/b]x dil factor x 900 /1000  (mg/900ml) | % released = (conc./original strength) x 100 |
| 5 |  |  |  |  |
| 10 |  |  |  |  |
| 15 |  |  |  |  |
| 20 |  |  |  |  |
| 25 |  |  |  |  |
| 30 |  |  |  |  |

* **Plot the Time versus % release on graph paper and comment on the effect of the Viscosity on the drug released rate??**
* **Determine the t80% of the drug??**
* **Comment on the release of the drug from the products at 30 minute according to USP requirements** (For phosphate buffer pH 5.8 in water**)**

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**Department of Pharmaceutics Biopharmaceutics PHT -414**

**Lab # 6**

**Effect of the Formulation Factor on the Drug Dissolution**

**DISSOLUTION TEST FOR PARACETAMOL TABLETS, WITH DIFFERENT FORMULATIONS (U.S.P. 1995)**

***Conditions:***

* + Apparatus : II (paddle)
  + Medium : 900 ml of phosphate buffer pH 5.8
  + Temp. : 37 ± 0.5 oC
  + Speed : 50 rpm
  + Time : 45 min.

***Procedure:***

1. Place one tablet in the vessel, and then start the apparatus at the above conditions.
2. At specified time intervals (5, 10, 15, 20, 25, 30, and 45 min) withdraw **1 ml** sample through a Millipore filtration unit (polyethylene tube with a cotton), and place the sample in a test tube. Replace the withdrawn sample with **1 ml** fresh phosphate buffer kept at 37 ± 0.5 o C.
3. Dilute **1 ml** of the collected sample to **25 ml** (dilution factor = 1:25) with fresh phosphate buffer (in a volumetric flask) , mix well.
4. Read the absorbance for the diluted samples at 243 nm against a blank of phosphate buffer.
5. Calculate the concentration of paracetamol released (from the LR equation of the line obtained by plotting the standard calibration curve) and express this conc. As a percentage of the labeled amount.
6. Plot the dissolution curve of paracetamol (% released vs.time).
7. From the dissolution curve, determine the time required for 80% of the labeled

**USP stated that:** the tablet should release not less than 80% of its content within 30 minutes and not less than 90% of its content within 45 minutes. (For immediate release tablets)

**Results of the dissolution --------------------------:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time  (min) | Abs.  at 243 nm | Dilution factor =  ( total vol / vol taken from the sample) | Conc. =[(abs-a)/b]x dil factor x 900 /1000  (mg/900ml) | % released = (conc./original strength) x 100 |
| 5 |  |  |  |  |
| 10 |  |  |  |  |
| 15 |  |  |  |  |
| 20 |  |  |  |  |
| 25 |  |  |  |  |
| 30 |  |  |  |  |
| 45 |  |  |  |  |

**Results of the dissolution of ------------------------:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time  (min) | Abs.  at 243 nm | Dilution factor =  ( total vol / vol taken from the sample) | Conc. =[(abs-a)/b]x dil factor x 900 /1000  (mg/900ml) | % released = (conc./original strength) x 100 |
| 5 |  |  |  |  |
| 10 |  |  |  |  |
| 15 |  |  |  |  |
| 20 |  |  |  |  |
| 25 |  |  |  |  |
| 30 |  |  |  |  |
| 45 |  |  |  |  |

* **Plot the Time versus % release on graph paper and comment on the effect of the Different Formulation factor on the drug released rate??**
* **Determine the t80% of the drug??**

Best Wishes

Student Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Student Number: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

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| **Lab. Number** | **Date** | **Mark** | **Signature** |
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