HPLC

Advanced Multiple-Choice Practice

15 Very Difficult Questions

Theory, Interpretation, Instrumentation & Calculations

Experiment 9
Chromatography (HPLC)

Instruction: Circle the single best answer for each question.

Answer key and model solutions are placed at the end of the file.

Section I - Theory & Interpretation (Questions 1-12)

Question 1. A mixture contains compounds X and Y. Compound X has a much greater affinity for the stationary phase, whereas Y has a greater affinity for the mobile phase. Which statement is most accurate?

- ○ A) X will generally migrate faster and elute before Y
- ○ B) Y will generally migrate faster and elute before X
- ○ C) Both compounds must have identical retention times
- ○ D) Separation occurs only if the mobile and stationary phases have identical affinities

Question 2. Which combination correctly distinguishes analytical chromatography from preparative chromatography?

- ○ A) Analytical -> purification and collection; Preparative -> qualitative identification only
- ○ B) Analytical -> determine sample composition qualitatively and quantitatively; Preparative -> purify and collect components
- ○ C) Analytical -> uses only a gas mobile phase; Preparative -> uses only a liquid mobile phase
- ○ D) Analytical -> requires HPLC; Preparative -> requires TLC

Question 3. In normal-phase adsorption chromatography, which analyte would be expected to have the longest retention?

- ○ A) A strongly polar analyte interacting with polar silica
- ○ B) A strongly non-polar analyte interacting with polar silica
- ○ C) A polar analyte carried by a highly polar stationary phase but having no interaction with it
- ○ D) The analyte with the weakest adsorption to the stationary phase

Question 4. A reverse-phase HPLC system uses a C18 stationary phase and a water-methanol mobile phase. Three analytes have increasing hydrophobicity: P < Q < R. What is the most likely elution order?

- ○ A) R -> Q -> P
- ○ B) P -> Q -> R
- ○ C) Q -> R -> P
- ○ D) All elute simultaneously because C18 is non-polar

Question 5. Which statement most accurately describes partition chromatography?

- ○ A) Separation depends only on adsorption onto a solid surface
- ○ B) Separation depends on the distribution of a solute between a liquid mobile phase and a liquid stationary phase coated on a solid support
- ○ C) Separation depends exclusively on molecular size
- ○ D) Separation requires covalent binding of the analyte to the stationary phase

Question 6. According to the lecture, which configuration correctly describes an anionic stationary phase / anionic exchanger?

- ○ A) Negatively charged resin that attracts anions
- ○ B) Positively charged resin that attracts anions
- ○ C) Positively charged resin that attracts cations
- ○ D) Uncharged porous resin that separates ions according to molecular size

Question 7. Three molecules are separated using size-exclusion chromatography:
- A: extremely large
- B: intermediate size
- C: extremely small

Assuming pore size allows C to penetrate extensively but excludes A, which retention pattern is expected?

- ○ A) C elutes first; A elutes last
- ○ B) A elutes first; C has the longest retention time
- ○ C) B elutes first; A and C elute together
- ○ D) Retention depends primarily on polarity, not size

Question 8. In affinity chromatography, a target analyte is strongly bound to an immobilized ligand while all other components have passed through. Which manipulation is specifically described as a method for subsequently eluting the retained target?

- ○ A) Increasing particle size only
- ○ B) Changing pH or ionic strength
- ○ C) Replacing the stationary phase with a gas
- ○ D) Eliminating the mobile phase completely

Question 9. Why can HPLC provide markedly better separation than conventional gravity-flow column chromatography?

- ○ A) Larger stationary-phase particles decrease interaction and therefore sharpen peaks
- ○ B) Very small particles provide greater surface area and interaction, while high pressure drives the mobile phase through the tightly packed column
- ○ C) HPLC eliminates the stationary phase and relies only on high pressure
- ○ D) High pressure decreases contact between the analyte and stationary phase

Question 10. Which sequence best represents the logical movement of the sample through the main HPLC system?

- ○ A) Injector -> Reservoir -> Detector -> Pump -> Column -> Recorder
- ○ B) Reservoir -> Pump -> Injector -> Column -> Detector -> Data system
- ○ C) Pump -> Detector -> Column -> Injector -> Reservoir -> Recorder
- ○ D) Reservoir -> Column -> Pump -> Detector -> Injector -> Recorder

Question 11. During an HPLC run, the baseline becomes unstable because dissolved gas is present in the eluent. In a separate experiment, retention behavior becomes poorly reproducible because column temperature fluctuates. Which pair of components directly addresses these two problems, respectively?

- ○ A) Injector and detector
- ○ B) Degasser and column heater
- ○ C) Pump and recorder
- ○ D) Autosampler and solvent reservoir

Question 12. The caffeine experiment uses all of the following conditions EXCEPT:

- ○ A) Water : Methanol = 60 : 40
- ○ B) Reverse-phase ODS column
- ○ C) Flow rate = 1 mL/min
- ○ D) UV detection = 254 nm

Section II - Calculations (Questions 13-15)

Question 13. An unknown caffeine powder is prepared exactly according to the experiment:

- 25 mg powder is dissolved to 50 mL.
- Then 5.00 mL of this solution is diluted to 100 mL.

Assuming the powder is 100% caffeine, what is the theoretical caffeine concentration in the final solution, and what is the dilution factor of the second dilution?

- ○ A) 0.500 mg/mL; DF = 10
- ○ B) 0.0500 mg/mL; DF = 20
- ○ C) 0.0250 mg/mL; DF = 20
- ○ D) 0.0125 mg/mL; DF = 40

Question 14. The caffeine calibration curve shown in the experiment follows:

Y = 1956.4X + 3409

where Y is the measured peak area. If the unknown caffeine sample produces a peak area of 101,229, what concentration X is obtained from the calibration equation?

- ○ A) 40.0
- ○ B) 45.0
- ○ C) 50.0
- ○ D) 52.5

Question 15. **A caffeine sample was prepared according to the experiment. From the calibration analysis, the final diluted solution is determined to contain:**

0.0235 mg/mL caffeine

The original solution volume was 50 mL, and the second dilution factor was 20. If the theoretical amount of powder weighed was 25.0 mg, what is the caffeine purity?

- ○ A) 89.0%
- ○ B) 92.0%
- ○ C) 94.0%
- ○ D) 98.0%

Answer Key & Model Solutions

Q1 B	**Q2** B	**Q3** A	**Q4** B	**Q5** B
Q6 B	**Q7** B	**Q8** B	**Q9** B	**Q10** B
Q11 B	**Q12** D	**Q13** C	**Q14** C	**Q15** C

Question 1 - B

Y has greater affinity for the mobile phase, so it generally migrates faster and elutes before X.

Question 2 - B

Analytical chromatography determines sample composition qualitatively and quantitatively, whereas preparative chromatography is used to purify and collect components.

Question 3 - A

In normal phase, the stationary phase is polar. A strongly polar analyte interacts most strongly with polar silica and therefore shows the longest retention.

Question 4 - B

In reverse-phase HPLC, the stationary phase is non-polar. The least hydrophobic analyte P elutes first, while the most hydrophobic analyte R is retained longest.

Question 5 - B

Partition chromatography separates solutes according to their distribution between a liquid mobile phase and a liquid stationary phase coated on a solid support.

Question 6 - B

The lecture describes an anionic exchanger as a positively charged resin that attracts anions.

Question 7 - B

Very large molecules are excluded from the pores and elute first. Very small molecules enter the pores extensively and therefore have the longest retention time.

Question 8 - B

Bound analytes in affinity chromatography can be eluted by changing conditions such as pH or ionic strength.

Question 9 - B

Small packing particles create a larger surface area and more interaction, while high pressure drives the mobile phase through the tightly packed column.

Question 10 - B

The main flow path is: reservoir -> pump -> injector -> column -> detector -> data system.

Question 11 - B

A degasser removes dissolved gases that destabilize the baseline, while a column heater maintains consistent column temperature for repeatable retention behavior.

Question 12 - D

The caffeine method uses UV detection at 275 nm, not 254 nm. The method also uses water:methanol 60:40, a reverse-phase ODS column, and a 1 mL/min flow rate.

Question 13 - C

Step 1: C1 = 25 mg / 50 mL = 0.5 mg/mL.
Step 2: C2 = (0.5 mg/mL x 5 mL) / 100 mL = 0.025 mg/mL.
Dilution factor: DF = 100 / 5 = 20.
Final answer: 0.0250 mg/mL; DF = 20.

Question 14 - C

101,229 = 1956.4X + 3409
X = (101,229 - 3409) / 1956.4 = 50.0.
Final answer: 50.0.

Question 15 - C

Actual amount: 0.0235 mg/mL x 50 mL x 20 = 23.5 mg.
Purity: (23.5 mg / 25.0 mg) x 100 = 94.0%.
Final answer: 94.0%.