you-try-it-02.xlsx
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For use with:
Brian M. Tissue, *Basics of Analytical Chemistry and Chemical Equilibria,* (John Wiley: New York, 2013).
http://www.achem.org

Worksheets in this file

notes	This page with background information.
2.A sample-prep	Tracking dilution in multistep procedures.
2.B single-extraction	Determining extraction fractions for single extractions.
2.C multiple-extractions	Determining extraction fractions for single extractions.
2.D extraction-volume	Determining optimum extraction conditions to minimize extractant volume.
2.E percent-recovery	Overall extraction efficiency in a multistep procedure.

Background
Refer to Chapter 2 in the text for equations and explanations.
Each worksheet has instructions in the blue shaded box.
For exercises 2.2, 2.3, and 2.4 you can use extraction.xlsx rather than writing your own formulas.
For step-by-step help see you-try-it-02guide.pdf.

You-Try-It 2.A **Sample Preparation**

Two sample preparations for vitamin tablets are listed in Procedures 2.A.1 and 2.A.2.
Table 2.A lists the amount of each vitamin in a tablet and the conversion factors for vitamin units (IU).
The table also provides formula weights, but these are not needed to answer the questions.
The internal standards are deuterated versions of the analytes.
The details of the actual analysis are omitted, as they are not necessary to answer the questions.

1. Calculate the ppm concentrations of vitamins A, D, E and K in the final extract solution for Procedure 2.A.1.
2. Calculate the ppm concentrations of vitamins A and E in the final extract solution for Procedure 2.A.2.

Table 2.A	each tablet contains:		1 IU is the biological equivalent of:			f.w. (g/mol)
Retinyl Acetate	Vitamin A	5000	IU	0.30	µg	328.490
Ergocalciferol	Vitamin D	400	IU	0.025	µg	396.650
α-Tocopheryl Acetate	Vitamin E	30	IU	1000	µg	472.743
Phylloquinone	Vitamin K	25	µg	---		450.700

Initial Preparation

grind 20 tablets to a fine powder using a mortar and pestle (each tablet is 1.5 g)
measure weights to 0.01 g unless specified otherwise.

Procedure 2.A.1

Measurement of Retinyl Acetate, α-Tocopheryl Acetate, Ergocalciferol, and Phylloquinone (Phytonadione).
Dissolve a 0.60 g portion of the powder in EDTA solution at 45 °C.
Add internal standard solutions containing retinyl acetate-d6, vitamin K1-d4, and vitamin D2-d3, respectively.
(The internal standards are measured simultaneously without affecting the analyte measurement.)
Place in an ultrasonicating bath for 10 min.
Extract analytes into hexane by shaking overnight. Perform the extraction five times.
Combine hexane extracts, add pure hexane to make up to 50.0 mL in a volumetric flask, mix.
Analyze solution by liquid chromatography/mass spectrometry (LC/MS).

sample mass:	
tablet mass:	
extract volume:	

analyte	analyte per g	analyte amount	analyte µg	analyte concentration
vitamin A				
vitamin D				
vitamin E				
vitamin K				

Procedure 2.A.2

Measurement of Carotenoids, Retinyl Acetate, and α-Tocopheryl Acetate

Dissolve a 2 g portion of the powder in dilute hydrochloric acid and ultrasonicate at 37 °C for 25 min.
Add internal standard solution (δ-tocopherol) and ultrasonicate an additional 5 min.
Extract analytes into hexane by shaking overnight.
Perform at least three subsequent hexane extractions (1 h on the shaker) until organic layer is colorless.
Combine hexane layers with additional pure hexane in a 50.0 mL volumetric flask and mix.
Remove 10.0 mL of extract, evaporated to dryness under nitrogen, and reconstituted in 1.0 mL of ethanol.
Analyze by liquid chromatography with UV-vis absorbance detection.

sample mass:	
tablet mass:	
extract volume:	
extract fraction:	

analyte	analyte per g	analyte amount	analyte µg	analyte concentration
vitamin A				
vitamin E				

Procedures adapted from:
NIST Analyses for Carotenoids and Fat-Soluble Vitamins
NIST SRM 3280 Certificate of Analysis
https://www-s.nist.gov/srmors/view_cert.cfm?srm=3280

You-Try-It 2.B **Single Liquid-Liquid Extraction**

Table 2.B.1 and 2.B.2 give log*P* values for a series of solutes for an octanol-water extraction.
Determine the extraction efficiency for a single extraction using an equal volume of the organic solvent.
The water sample volume is 10 mL.

1. Write a formula to convert the log*P* values to K_D'.
2. Write a formula to find the fraction remaining in the aqueous phase, $\alpha_{S(aq)}$.
3. Convert $\alpha_{S(aq)}$ to $\alpha_{S(org)}$.

Table 2.B.1

compound	log*P*	K_D'	V_{aq} (mL)	V_{org} (mL)	*n*	$\alpha_{S(aq)}$	$\alpha_{S(org)}$
1-hexanol	2.03		10	10	1		
benzene	2.13		10	10	1		
1-octanol	3.07		10	10	1		
cyclohexane	3.44		10	10	1		
hexane	4.00		10	10	1		
octane	5.15		10	10	1		

Table 2.B.2

compound	log*P*	K_D'	V_{aq} (mL)	V_{org} (mL)	*n*	$\alpha_{S(aq)}$	$\alpha_{S(org)}$
methanol	-0.74		10	10	1		
ethanol	-0.30		10	10	1		
acetone	-0.24		10	10	1		
acetic acid	-0.17		10	10	1		

log*P* values from:
CRC Handbook of Chemistry and Physics

You-Try-It 2.C **Sequential Liquid-Liquid Extractions**

Table 2.B.2 from worksheet 2.B is copied here.
Copy the formulas for alpha that you entered on worksheet 2.B.
Check that the results do not change on copying the formulas.

Determine the number of sequential extractions that are necessary to achieve the following efficiencies.
Again use equal volumes of the aqueous and organic phases.

1. Extract > 95 % of each solute to the organic phase.
2. Extract > 99 % of each solute to the organic phase.

Table 2.C.1 Extract > 95 %

compound	log*P*	K_D'	V_{aq} (mL)	V_{org} (mL)	*n*	$\alpha_{S(aq)}$	$\alpha_{S(org)}$
methanol	-0.74	0.18	10	10			
ethanol	-0.30	0.50	10	10			
acetone	-0.24	0.58	10	10			
acetic acid	-0.17	0.68	10	10			

Table 2.C.2 Extract > 99 %

compound	log*P*	K_D'	V_{aq} (mL)	V_{org} (mL)	*n*	$\alpha_{S(aq)}$	$\alpha_{S(org)}$
methanol	-0.74	0.18	10	10			
ethanol	-0.30	0.50	10	10			
acetone	-0.24	0.58	10	10			
acetic acid	-0.17	0.68	10	10			

You-Try-It 2.D **Optimizing a Liquid-Liquid Extraction**

An accepted method uses diethyl ether to extract acetic acid from water samples.
The extraction calculations in Table 2.D.1 show that the total organic volume is high, e.g.:
≥ 200 mL for most values of n .
You want to determine if changing the extraction solvent to 1-octanol can reduce n and the amount of solvent.
The log$P$ and distribution constants for acetic acid are given in the top table to the left.
Determine optimal conditions for extracting acetic acid from a water sample with 1-octanol.
Constraints:
the water sample is 10 mL
the extraction should transfer > 99.5 % of the acetic acid
the total extractant volume should be less than ≈ 150 mL
doing more than 10 extractions is too time consuming

1. Set up Table 2.D.2 for 1-octanol similar to the one for diethyl ether.
   Hints:
   Vary n (3,4,5,...) and $V_{S(org)}$ (50, 100, 150 mL,...) to find extraction efficiencies.
   For larger n , refine $V_{S(org)}$ in smaller increments to find conditions that give > 99.5 extraction.

2. Determine the optimal conditions from the results.

acetic acid distribution constants

organic phase	log$P$	K_D'
diethyl ether	-0.36	0.44
1-octanol	-0.17	0.68

V_{aq} (mL)	
10	mL

Table 2.D.1 **diethyl ether**

V_{org} (mL)	n	$\alpha_{S(aq)}$	$\alpha_{S(org)}$	V_{total} (mL)
100	3	0.006	0.994	300
105	3	0.006	0.994	315
110	3	0.005	0.995	330
60	4	0.006	0.994	240
65	4	0.005	0.995	260
70	4	0.004	0.996	280
40	5	0.006	0.994	200
42	5	0.005	0.995	210
30	6	0.007	0.993	180
32	6	0.005	0.995	192
25	7	0.006	0.994	175
26	7	0.005	0.995	182
20	8	0.007	0.993	160
21	8	0.005	0.995	168
17	9	0.007	0.993	153
18	9	0.005	0.995	162
15	10	0.006	0.994	150
16	10	0.005	0.995	160

Table 2.D.2 **1-octanol**

V_{org} (mL)	n	$\alpha_{S(aq)}$	$\alpha_{S(org)}$	V_{total} (mL)

diethyl ether log*P* from:
Manni, G.; Caron, F.
Calibration and determination of volatile fatty acids in wastel□eachates by gas chromatography
Journal of Chromatography A, 690 (1995) 237-242

You-Try-It 2.E **Percent Recovery and Internal Standard Calibration**

A sample preparation procedure, calibration data, and analytical results are listed to the left.
The response of the chromatography analysis is linear in the concentration range of the analyte.
The measurement procedure is identical for the sample and the calibration standards.
Other details of the chromatographic analysis are not important.

Analysis Information and Assumptions:

the percent recovery of the analytes is expected to be the same as the internal standards
the analytes and internal standard have the same sensitivity in the analytical measurement

Use the data for the following calculations.

1. Determine the percent recovery of the internal standard.
2. Determine the concentration of the two unknowns in the plasma sample.

Sample cleanup for norepinephrine and epinephrine in plasma

Position a 1 mL SPE tube with carboxylic acid stationary phase on a vacuum manifold.
Condition SPE column with 0.5 mL of 0.5 M HCl.
add 1 mL water to remove excess acid.
Mix 500 µL plasma with 500 µL water and load on SPE column.
Adjust vacuum manifold to pass sample through the tube at 0.25 mL/min.
Wash with two 1 mL portions of water.
Add internal standard (400 pg dihydroxybenzylamine in 50 µL water) and 250 µL water dropwise.
Elute analytes with 250 µL 0.2 M perchloric acid dropwise.
Collect eluate in a graduated vial and add water to make up to exactly 500 µL.
Analze by liquid chromatography with electrochemical detection.

Dihydroxybenzylamine Calibration Data:

conc (pg/500 µL)	signal (pA)	LINEST
0	2.5	
125	51.3	
250	124.8	
500	232.1	

Measurement Results:

analyte	signal (pA)	conc (pg/500 µL)	percent recovery
internal std	179		%
norepinephrine	177		
epinephrine	83.5		

	plasma conc (pg/mL)
norepinephrine	
epinephrine	

procedure adapted from:

Supelco Application Note 66
Simple Solid Phase Extraction and HPLC Analysis of Catecholamines in Plasma