COSMETIC CREAMS
Identification and determination of parabens

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English, Hungarian

Summary
To conserve the quality of food, pharmaceuticals and cosmetics for a relatively long time, different preservatives are added to them. Parabens (4-hydroxybenzoic acid esters), as oestrogenic chemicals, can protect against micro-organisms like bacteria and moulds. You are asked to identify and quantitatively determine the frequently used methyl-, ethyl-, propyl-, butyl-parabens, 2-phenoxyethanol, and 1-phenoxypropan-2-ol preservatives in cosmetic creams (e.g. Sanex).
Students work in groups to extract the parabens from the cream and then identify, by thin layer chromatography (TLC), and quantitatively determine, using high performance liquid chromatographic (HPLC) separation and UV detection. The tasks include: evaluating the results, calculating the cost of the methods and communicating with a 'customer' of the students' laboratory or others (e.g. the public, press, other students).

Activity type **G**
Use of scientific knowledge and understanding to solve problems
Working in teams to solve problems
Communication
Resource/ budget management

Techniques
Solid-liquid and liquid-liquid extraction
Thin layer chromatography
High performance liquid chromatography

Field
Cosmetics

Time
Practical lessons: 300 minutes
Theory lessons: 90 minutes
Work in class: 90 minutes
Out of class time: 60 minutes

StandardBase procedures
Identification of parabenes in Sanex cream using TLC
Determination of methylparabene in Sanex cream using HPLC

StandardBase techniques
Thin layer chromatography
High performance liquid chromatography

Other resources
TechnoMatch, Drachten, The Netherlands: www.technomatch.nl
Agilent, the Netherlands: www.agilent.com
See also in the activity.

Student's document

COSMETIC CREAMS: Identification and determination of parabens

Many thousands of cosmetic, food and pharmaceutical products would deteriorate or lose their perfume, flavour and/or quality in a short time without any preservative. Parabens are synthetic, widely used preservatives, which in high concentration can cause allergic reactions and skin rashes, and can disrupt the hormonal system through oestrogenic activity [Darbre PD. (2006): Environmental oestrogens, cosmetics and breast cancer. Best Pract. Res. Clin. Endocrinol. Metab. 20, 121-143]. Parabens are used in non-hazardous low concentrations e.g. in cosmetic creams like Sanex.
The measurement of various compounds is a good challenge for the analyst - choosing the best (cost, time, energy and performance) extraction, separation and detection method, and considering its complexity.

Your brief

You are an analyst working in a group which has been asked to identify and determine some parabens used as preservatives in Sanex cream. You have to know about the basics of the liquid extraction, thin layer chromatographic and high performance liquid chromatographic separation methods. You must also estimate their cost on the basis of the material demand (chemicals, mobile and stationary phases), and communicate your results to fellow students, 'customers' and other people.

Your investigation

You will work in a group of four. Two members of the group will identify the parabens with TLC, the other two will measure them with HPLC. You will be provided with:

- helpful websites
- information sheets for experiments: **Student sheet***: Identification of parabens in cosmetic creams* and **Student sheet***:Quantitative determination of parabens in cosmetic creams*
- **Data sheets** *Identification by TLC experiment* and *RP-HPLC investigation* for evaluating results

This activity is about communication and management of resources and budget. Your teacher will help you to manage them. Work as a team and follow these instructions:

- Search information about the role and use of parabens in different food and cosmetics, especially in Sanex cream.

You can find information at http://www.sanex.net/ and in these publications:
- N. de Kruijf, M.A.H. Rijk, L.A. Pranato-Soetardhi and A. Schouten (1987): Determination of preservatives in cosmetic products I: Thin-layer chromatographic procedure for the identification of preservatives in cosmetic products (J. Chromatography 410, 395-411).
- N. de Kruijf, M.A.H. Rijk, L.A. Pranato-Soetardhi and A. Schouten, (1989). Determination of preservatives in cosmetic products II. High-performance liquid chromatographic identification (J. Chromatography 469, 317-398).

- During the experiments you will use solid-liquid and liquid-liquid extractions, thin layer chromatography and high performance liquid chromatography. Look up the fundamental principles of these separation techniques.

Some useful websites are:
http://www.chem.wisc.edu/courses/342/Fall2004/TLC.pdf
http://www.lipidlibrary.co.uk/topics/tlc/file.pdf
http://siggy.chem.ucla.edu/VOH/136/TLC.pdf
http://www.chromatography-online.org/Principles/TLC/Sample-Application/rs68.html
Note: download the "Principles and Practice of Chromatography" (it is free, but you have to register). Pages 48-67 are especially recommended.
http://www.ionsource.com/tutorial/chromatography/rphplc.htm
http://www.waters.com/WatersDivision/ContentD.asp?watersit=JDRS-6VRHBW&WT.svl=1

- Read through the experimental section carefully. Look at the list of equipment and materials you need and find their prices in catalogues.

Use book catalogues or websites:
http://www.merck.de/servlet/PB/menu/1001723/index.html
Use the ChemDat online.
http://www.sigmaaldrich.com/Local/SA_Splash.html

A risk assessment must be carried out before starting practical work.

- After sharing out the work, and thinking over what data you need (e.g. don't forget to write down the mass of the cream!), you can start the experimental work.
- For the evaluation of your results, use **Student sheet***: Identification by TLC experiment* and **Student sheet***: RP-HPLC investigation* as a guide.
- Share and discuss the results of both methods and compare them. Prepare your report (to 'customers', the public, press, fellow students etc.).

Your findings
Prepare a report to other students or to others outside the school who may have an interest in your findings.

Summarise the role of parabens in the cosmetic products investigated.

Try to summarise the differences between TLC and HPLC using the correct terms! Think about, for example, how enclosed the systems are, sampling, the way the sample moves through the mobile phase, the speed of the mobile phase, the detection methods.

Developing an HPLC method needs lengthy laboratory work. How can you identify factors that interfere with your data? How would you deal with them?

Discuss the results from both methods and their estimated cost. Remember that the cost depends on the purity (e.g. chromatographic grade mobile phase and high purity parabens are not needed for TLC) and the quantity of chemicals (e.g. HPLC requires more mobile phase than TLC).

Discuss when you would use each method. Think about their cost, time-demand and precision.

Student self assessment
This activity was about the use of scientific knowledge and understanding to solve problems, working in teams, communication and managing resources and budgets.

Answer, in 50-100 words, the following questions:

What scientific skills and knowledge did you acquire and how effectively could you use them?

How successfully did you help to improve the work of the group? Give some advantages of teamwork using examples.

What ways of communicating did you use and how effectively did you use them?

How did you manage resources and budgets?

Student sheet: Identification of parabens in cosmetic creams

(based on StandardBase procedure)

EXTRACTION FOR TLC IDENTIFICATION

Materials and equipment

- acetone
- calcium chloride dehydrate
- diethyl ether
- hydrochloric acid (4 mol/L)
- potassium hydroxide solution (4 mol/L)
- analytical balance
- 50 mL glass tube with screw cap
- water bath for 60 ^{o}C
- pH indicator paper
- filter paper
- conical flask
- separating funnel
- sample vial (5 mL)

Procedure

Weigh 1.0 g of Sanex cream into a 50 mL glass tube with screw cap. Add four drops of hydrochloric acid solution and 40 mL of acetone. Put the closed tube in a water bath kept at 60 °C, to facilitate the extraction of the parabens into the liquid phase. Leave the tube in the water bath for five minutes, occasionally shaking vigorously.
Adjust the pH of the solution with hydrochloric acid (it is not allowed to be above pH=3) measuring with pH indicator paper. Shake briskly again for one minute. Cool the solution to room temperature with cold running water. Filter through a filter paper. Transfer 20 mL of the filtrate and 60 mL water into a 200 mL conical flask and mix them. Adjust the pH of the mixture to approximately pH=10 with potassium hydroxide solution, monitoring with pH indicator paper.
Add 1 g calcium chloride dehydrate and shake vigorously. Filter the solution through a filter paper into a 250 mL separating funnel containing 75 mL diethyl ether and shake vigorously for one minute. When the layers are separated, collect the aqueous layer into a 200 mL conical flask and adjust its pH to approximately pH=2 with hydrochloric acid solution, using pH indicator. Subsequently, add 10 mL diethyl ether and shake vigorously for one minute. Allow the phases to separate and transfer approximately 2 mL of this second diethyl ether layer into a 5 mL sample vial. (The diethyl ether layer of the first extraction is not needed for the further investigation.)

TLC INVESTIGATION

Materials and equipment

- acetic acid, glacial
- n-pentane
- methanolic solutions of
  - methyl 4-hydroxybenzoate (methylparaben)
  - ethyl 4-hydroxybenzoate (ethylparaben)

  - n-propyl 4-hydroxybenzoate (propylparaben)
  - n-butyl 4-hydroxybenzoate (butylparaben)
- 0.2 g/100 mL methanolic solutions of
  - 2-phenoxyethanol
  - 1-phenoxypropan-2-ol
- TLC plates, 20 cm x 20 cm aluminium sheets precoated with 0.25 mm silica gel containing a fluorescing agent, if possible, with concentration zone
- oven, capable of maintaining up to 120 °C
- microsyringe
- developing chamber (unsaturated)
- air stream source (e.g. hair dryer)
- UV lamp (λ = 254 nm)

Procedure

Apply 10 µL of each reference solution and 100 µL of the sample solution onto the start line (in the concentration zone) on the previously heated (3 h, 120 °C) TLC plate. Drying can be speeded up by using a stream of cold air.

Mix the mobile phase [n-pentane-glacial acetic acid (88:12 *V*/*V*; made e.g. by mixing 88 ml of n-pentane and 12 ml of glacial acetic acid)] and transfer 100 mL into the separation chamber. Immediately place the TLC plate in the unsaturated chamber and develop at room temperature until the solvent front has run about 15 cm from the base line (the development distance is important for separation of the 6 parabens). Dry the developed layer in a stream of hot air. Mark the position of the spots under UV light and evaluate the plate.

For evaluation of the results, use **Student sheet***: Identification by TLC experiment*.

Student sheet:
Quantitative determination of parabens in cosmetic creams
(based on StandardBase procedure)

EXTRACTION FOR HPLC DETERMINATION

Materials and equipment
- ethanol, absolute
- sulfuric acid (2 mol/L)
- 100 mL glass tubes with screw cap
- boiling chips, carborundum
- analytical balance
- water bath for 60 °C
- filter paper
- sample vial (5 mL)

Procedure
Weigh 1.0 g of Sanex cream into a 100 mL glass tube. Add 1.0 mL sulfuric acid solution, 50.0 mL ethanol-water mixture (9:1 *V*/*V*; use a solvent mixture made in the following way: 90 ml absolute ethanol + 10 ml water) and about 1 g of boiling chips to the tube. Shake the closed tube briskly, for at least one minute, to suspend the sample in the solvent mixture. Put the tube in a water bath kept at 60 °C ± 1 °C. Leave for five minutes to facilitate the extraction of the parabens into the liquid phase. Immediately cool the tube in a stream of cold water and store the extract in the refrigerator for one hour. Filter the extract using a filter paper. Transfer approximately 2 mL of the filtrate into a 5 mL sample vial. Store the extracts in the refrigerator and perform the HPLC determination within 24 hours.

HPLC INVESTIGATION

Materials and equipment
- acetonitrile
- ethanol, absolute
- methanol
- sulfuric acid
- tetrahydrofuran
- stock solution containing the following preservatives (in ethanol/water 9:1 *V*/*V*;):
  - 0.05 g/100 mL methyl 4-hydroxybenzoate (methylparaben)
  - 0.05 g/100 mL ethyl 4-hydroxybenzoate (ethylparaben)
  - 0.05 g/100 mL n-propyl 4-hydroxybenzoate (propylparaben)
  - 0.05 g/100 mL n-butyl 4-hydroxybenzoate (butylparaben)
  - 0.2 g/100 mL 2-phenoxyethanol
  - 0.2 g/100 mL 1-phenoxypropan-2-ol
- 50 mL volumetric flasks
- syringe filter (nylon, PVDF, 0.45 µm porosity)
- HPLC with UV-detector (λ=280 nm) and reversed phase analytical column (Nucleosil 5C18 or equivalent, stainless steel, 25 cm x 4.6 mm) with 20 µL loop
- microsyringe

Procedure

Prepare 6 standard preservative solutions. Transfer 0.5, 1, 2, 5, 10 and 20 mL stock solution respectively into 50 mL volumetric flasks. To each flask, add 1 mL sulfuric acid and fill up to the line with ethanol-water (9:1 *V*/*V*) mixture. (Use a solvent mixture to fill up each flask made e.g. as follows: 270 ml absolute ethanol + 30 ml water.) Prepare these solutions fresh.

For HPLC separation, mix and filter the mobile phase [tetrahydrofuran-water-methanol-acetonitrile (5:60:10:25 *V*/*V*/*V*/*V*)]. Use, for example, a solvent mixture made as follows: 5 ml tetrahydrofuran + 60 ml water + 10 ml methanol + 25 ml acetonitrile. Adjust the flow rate of the mobile phase (1.5 mL min^{-1}) and the UV detector (λ= 280 nm). The injected volume should be 20 µL of each solution (standard and sample). In all cases, record the chromatogram and measure the peak areas or heights.

If the obtained worst R_s has not achieved 0.9, than you should change the composition of the mobile phase or choose a more efficient column.

For evaluation of the results, use **Student sheet***: RP-HPLC investigation*.

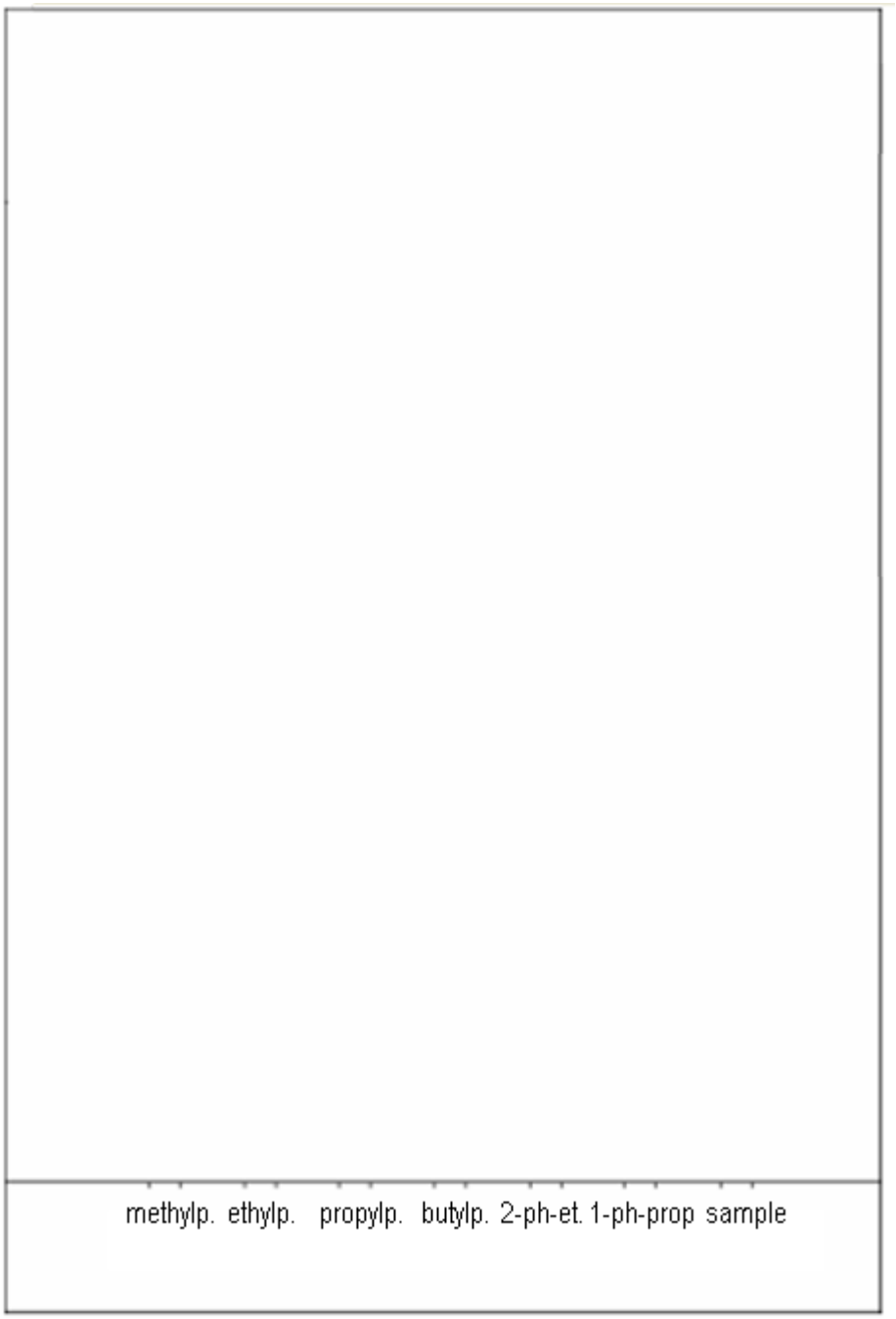
Student sheet: Identification by TLC experiment

Documenting the TLC separation
Draw the spots of the parabens to the exact area, as they are in the developed layer. The correct centre and size of the spots are important.
Don't forget to draw the eluent front.
You need the following distances (mark them on the drawing):

The distance between the origin (applying point) and the eluent front (mm):

h_o=_____mm

The distance between the origin and the centre of each spot (mm) [*h*]



Fill out the following table. Calculate the R_f value for each spot. Compare the spots obtained from the sample solution with those of the reference solutions with respect to their R_f values, as seen under UV radiation.

	h [mm] height of the spot	$R_f = h/h_o$ retardation factor	found in the cream? (Yes or No)
methylparaben			
ethylparaben			
propylparaben			
butylparaben			
2-phenoxyethanol			
1-phenoxypropan-2-ol			

Student sheet: RP-HPLC investigation

Paste here the chromatograms of a standard solution (containing all test substances) and the sample solution.
Chromatogram of a standard solution:

Chromatogram of the sample solution:

What is dead time (t_o) in HPLC? Mark it on the chromatogram of the standard solution.

t_o=_____min

You will also need the retention time (t_R) and the baseline width (w) of all examined parabens. Collect these parameters into the following table from the chromatogram of the standard solution. Calculate the reduced retention time (t_R'), the resolution (R_s), the selectivity (α), the number of theoretical plates (n) and the height of the theoretical plate ($HETP$) of the adjacent peaks using the following formulas:

$$t_R' = t_R - t_0$$

$$R_s = \frac{t_{R2} \quad t_{R1}}{\frac{w_1 + w_2}{2}} \quad \text{(if } t_{R2} > t_{R1}\text{)}$$

$$\alpha = \frac{t_{R2}'}{t_{R1}'}$$

$$n = 16\left(\frac{t_R}{w}\right)^2$$

$$HETP = \frac{L}{n}$$

	t_R	t_R'	R_s	α	n	*HETP*
methylparaben						
ethylparaben						
propylparaben						
butylparaben						
2-phenoxyethanol						
1-phenoxypropan-2-ol						

Write the average peak areas of the different parabens, measured in the chromatogram for the standard solutions, in the next table. Calculate the concentrations of the parabens in the standard solutions.

	Average peak areas					
Concentration of each parabens in the standard solution [mg L^{-1}]						
methylparaben						
ethylparaben						
propylparaben						
butylparaben						
Concentration of each parabens in the standard solution [mg L^{-1}]						
2-phenoxyethanol						
1-phenoxypropan-2-ol						

Fill in the following table. In Microsoft Excel, plot the average peak areas (*T*) for each of the parabens of the standard solutions against the concentrations (*c*) in mg L^{-1}. You can obtain the function between the peak areas and the

concentration by linear regression and the correlation coefficients. To find the concentrations of parabens in the sample solution, you have to substitute the average peak areas of the parabens of the sample solution into these functions. Add the methyl-, ethyl-, propyl-, butyl-parabens, 2-phenoxyethanol, and 1-phenoxypropan-2-ol mass fraction (w) of the Sanex cream in mg g^{-1}, using this formula:

$$w = \frac{c \cdot V}{10^3 \cdot m_0}$$

where m_o = the mass of the sample (the cream) in grams
V = volume of the solution (51 mL)

	function between T and c with R	average T of the sample solution	c in the sample solution [mg L^{-1}]	Mass fraction in the cream [mg g^{-1}]
methylparaben				
ethylparaben				
propylparaben				
butylparaben				
2-phenoxyethanol				
1-phenoxypropan-2-ol				

Teachers' document

Overview

In this activity, students experience some of the work of an analyst such as the purchase of equipment and materials, sample preparation and chromatographic identification and determination. Emphasis should be laid on correct written and verbal communication together with resource and budget management.

Developing students' skills

Laboratory work provides ample opportunities for students to cultivate skills such as:

- collecting, analysing and organising information
- deeper critical thinking skills to analyse data and errors
- problem-solving skills
- hands-on skills using a wide variety of instruments and techniques
- written and oral communication about ideas and information
- planning and organising activities
- effectively working alone and cooperatively in teams
- using working time efficiently
- handling and setting up apparatus smoothly
- keeping the working area tidy
- using mathematical ideas
- managing work with computers.

Organising and managing the class

In view of the large amount of practical work, students might need to be helped with their timing and workload.

When required, managing the budget may need help and guidance. Students need to know where they can find resources and the cost of materials and equipment. Catalogues of apparatus and chemical suppliers (books, Internet facility) must be available for the students. Attention should be given to the use of nomenclature, the difference in the price for buying in different quantities (mass, volume) and comparison with products from other companies.

The sources of hazards and mistakes should be discussed before the start of practical work. Depending on the level of experience of the students, they will need appropriate guidance on the correct use of equipment such as an analytical balance, separating funnel, HPLC (especially in setting the HPLC) and handling tools.

Session plan for teachers

Introduction and checking prior knowledge	30 minutes
Arranging students in groups to discuss and plan the work	30 minutes
Cost estimation	60 minutes
Carrying out the practical work	300 minutes
Evaluation, discussion and presentation of results	120 minutes

Technical notes
See the methods on the student sheets.

- **Preparation**

The HPLC method should be checked in advance. If the required separation is not achieved, a more efficient column should be used, or the mobile phase composition should be adjusted. To achieve the maximum detector signal, spectra of the given substances should be recorded, and the best wavelength should be chosen. Students must be told about the changed conditions. It should be explained to them that working with HPLC needs lengthy practical experience, and the arrangement of HPLC for students to use is therefore done for them by the teacher or technician.

The following test solutions should be made up in advance:

- 0.1 g/100 mL methanolic solutions of methylparaben, ethylparaben, propylparaben, and butylparaben
- 0.2 g/100 mL methanolic solutions of 2-phenoxyethanol and 1-phenoxypropan-2-ol
- stock solution containing the following preservatives (in ethanol/water 9:1 *V*/*V*):
  - 0.05 g/100 mL methyl 4-hydroxybenzoate (methylparaben)
  - 0.05 g/100 mL ethyl 4-hydroxybenzoate (ethylparaben)
  - 0.05 g/100 mL n-propyl 4-hydroxybenzoate (propylparaben)
  - 0.05 g/100 mL n-butyl 4-hydroxybenzoate (butylparaben)
  - 0.2 g/100 mL 2-phenoxyethanol
  - 0.2 g/100 mL 1-phenoxypropan-2-ol

To prepare the stock solution use a solvent mixture made, for example, in the following way: 90 ml absolute ethanol + 10 ml water; this stock solution is prepared in advance and is stable for 1 week if kept in a refrigerator.

- **Equipment and materials**

Materials and their CAS numbers

Name	**CAS number**
Acetic acid, glacial	64-19-7
Acetone	67-64-1
Acetonitrile	75-05-8
Calcium chloride dehydrate	10035-04-8
Diethylether	60-29-7
Ethanol, absolute	64-17-5
Hydrochloric acid	7647-01-0
Methanol	67-56-1
n-Pentane	109-66-0
Potassium hydroxide	1310-58-3
Sulfuric acid	7664-93-9
Tetrahydrofuran	109-99-9
Methyl 4-hydroxybenzoate (methylparaben)	99-76-3
Ethyl 4-hydroxybenzoate (ethylparaben)	120-47-8
n-Propyl 4-hydroxybenzoate (propylparaben)	94-13-3
n-Butyl 4-hydroxybenzoate (butylparaben)	94-26-8
2-Phenoxyethanol	122-99-6
1-Phenoxy-propan-2-ol	770-35-4

Equipment

- Normal laboratory equipment including distilled water, pH indicator paper, analytical balance and volumetric flasks

Extractions

- 50 and 100 mL glass tubes with screw cap
- separating funnel
- boiling chips, carborundum
- water bath for 60 °C
- filter paper

TLC identification

- TLC plates, 20 cm x 20 cm aluminium sheets precoated with 0.25 mm silica gel containing a fluorescing agent (e.g. Merck silica gel 60F254 No 1.05554)
- oven, capable of maintaining up to 120 °C
- microsyringe (10 and 100 µL)
- developing chamber (unsaturated)
- air stream source (e.g. hair dryer)
- UV lamp (λ=254 nm)

HPLC determination

- syringe filter (nylon, PVDF, 0.45 µm porosity)
- HPLC with UV-detector (λ=280 nm) and reversed phase analytical column (Nucleosil 5C18 or equivalent, stainless steel, 25 cm x 4.6 mm)
- microsyringe (50 or 100 µL)

TESTING PRIOR KNOWLEDGE

1. What is the role of parabens in cosmetics?
Ans.: preservatives, against micro-organism growth

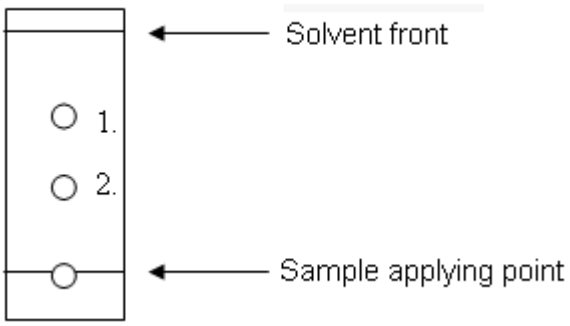
2. What is the fundamental concept of the separation method in liquid chromatography?
Ans.: the interactions between the mobile phase, the stationary phase and the substances

3. How can you increase the efficiency of a liquid-liquid extraction?
Ans.: change the extracting solvent; increase the volume of the extracting phase; extract more times

4. What is the driving force of the mobile phase in TLC?
Ans.: capillary action

5. What is the best deactivator of the silica gel layer? Think about why we heat it before use.
Ans.: H_2O

6. Determine the retention factors and the resolution on the developed silica gel TLC plate.



Ans.: $R_{f1} \approx 0.65$; $R_{f2} \approx 0.35$; $R_s \approx 3,1$

7. Sketch and label the main features of HPLC.
Ans.: mobile phase, solvent delivery system (pump), sample injector, pre-column, column, detector, recorder, waste or collect fractions.

Note: Correct answers are given in red. These need to be removed before the test is used.

TESTING PRIOR KNOWLEDGE

1. What is the role of parabens in cosmetics?

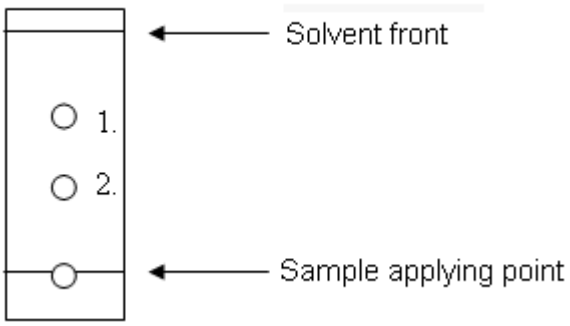
2. What is the fundamental concept of the separation method in liquid chromatography?

3. How can you increase the efficiency of a liquid-liquid extraction?

4. What is the driving force of the mobile phase in TLC?

5. What is the best deactivator of the silica gel layer? Think about why we heat it before use.

6. Determine the retention factors and the resolution on the developed silica gel TLC plate.



7. Sketch and label the main features of HPLC.