 Candidate no.: ……

No. of additional pages:……

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Faculty of Natural Sciences and Technology
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EXAMINATION IN COURSE KJ 2053
CHROMATOGRAPHY

THURSDAY, JUNE 2ND 2016,
09:00 - 13:00 (4 HOURS)

English

Answers for questions 1-8 are to be written into this questionnaire, which is delivered to the examination officer at the end of the examination
(Use of graphite pencil is discouraged, especially due to lack of a student copy of the answers. It is however also possible to write the answers on the usual examination sheets, keeping the copy sheets for yourself.)

If not enough space is available in the questionnaire for your answer(s) use the opposite side/back side, or separate answer sheets which then are returned together with the questionnaire. Reference to these separate sheets in the questionnaire for the questions where this is relevant.

Permitted aids: code D
B1-type calculator with empty memory
(as specified in NTNU's list of calculators approved for use in examinations at NTNU)
No other aids (i.e. printed or handwritten texts or notes) are allowed.

This test consists of fifteen - 15 - pages :
1 cover page, 1 front page (p. 1), 13 pages with 8 questions (p. 2-14).

Weighting factors for the questions are given for each of the question. A maximum of 103 ~~104~~ points may be obtained. Scores are calculated assuming a maximum score of 100 pts.

Sign.
……..
Rudolf Schmid
Responsible examiner

Examination text checked by:

Sign.
……
Dag Ekeberg

Deadline for the results to be announced: June 23rd 2016.

Question 1 : *(5 + 8 = 13 p.)*

1.a) (i) What is the relationship between k, t_R and t_0 ?
(1 p.)

(i) What are the lowest values possible for α and for R_S ?
(1 p.)

…… < α ….. < R_S

(1 p.) (iii) How do you quantify peak asymmetry in a chromatogram (give a formula, you may supplement, it with a sketch/figure) ?

(iv) Among the 12 symbols given below, identify the ones that express a measure of efficiency for a chromatographic column. (Ignore the other symbols.)
(2 p.) Give the full name of the symbols of interest here, and indicate, for each of them, whether increasing efficiency is expressed by an increasing numerical value or by a decreasing one.

t_R , R_F , TZ (SN), A_s , μ_{eof} , K_{av} , N_{eff} , I(x) , H , w_b , T_{iso} , ε°.

1.b) (i) Write the formula for the simplified "*van Deemter* equation" (3 terms) and name the terms in the equation:

(2 p.)

= + +

(ii) The last term is often sub-divided further, explain why and how (show these subdivided terms).

(2 p.)

(iii) The equation is simpler when it is used to describe open capillary columns. Show this simplified equation, describe the simplification, and explain why this is possible/reasonable.

(2 p.)

(iv) Illustrate the *van Deemter* equation graphically (use packed column gas chromatography as an example, specifiy x- and y-axes).

(2 p.)

Van Deemter plot for GC

Question 2 : *(5+3+7 = 16 p.)*

2.a) (i) What is meant by ”planar chromatography” ? List examples of such techniques.

(2 p.)

(ii) What are the advantages of planar chromatography when compared to column chromatography, HPLC ?

(2 p.)

(iii) Which is/are the most common retention parameter(s) used in planar chromatography ?

(1 p.)

2.b) (i) Draw a representative partial molecular structure of the surface of (untreated) silica (and name the important structural features on it).

(2 p.)

(ii) Name the chromatographic technique, that uses silica gel in combination with mobile phases consisting of dry organic liquids of low to intermediate polarity.

(1 p.)

2.c) (i) What is the most popular stationary phase (SP) for reversed-phase (RP-) HPLC ? Give specific name, and draw a representative (partial) molecular structure.
(3 p.)

(iii) Give a nam
e, and draw a structure of the stationary phase we used in our lab. experiments in GLC (and which is the most popular SP type in GLC).
(2 p.)

(iii) Name at least two stationary phases used in gas-solid chromatography (GSC, gas adsorption chromatography). Tell for each one, whether it is polar or non-polar.
(2 p.)

Question 3 : *(2+7 = 9 p.)*

3.a) Which GC technique (if any) can be used to separate the analytes given below:
(4 p) Tick as appropriate, comment if necessary,

Analyte	GSC	GLC	GC not applicable	Why (Comments) ?
methane and argon				
quinoline, benzophenone and 1-chloronaphthalene				
insulin and hemoglobin				
sucrose, glucose and glycerol				

3.d) (i) Briefly describe the injector and injection technique (procedure) for a split-less injection.

(3 p.)

(ii) what is/are the advantage(s) of using an autosampler instead of performing manual injections in gas chromatography ?

(2 p.)

Question 4 : *(3+6+5+1 =15 p.)*

4.a) (i) Name two methods, that represent liquid-liquid chromatography, LLC.
(1 p.)

(ii) Report the typical dimensions for a packed HPLC column (length / inner diameter / particle diameter) and the typical pressure limits of such a column.
(1 p.)

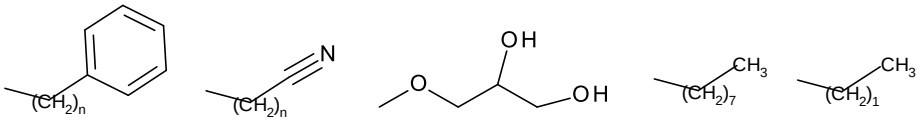
(iii) Is the most common HPLC capillary column type today a packed, a PLOT or a WCOT capillary column ?
(1 p.)

4.b (i) In normal-phase adsorption chromatography (NP-LSC), e.g. with untreated silica as the stationary phase, "activity" is an important parameter:
Explain what activity is, in LSC. How can the activity be regulated/adjusted ?
(3 p.)

(ii) Name two other materials than silica gel, that are used as stationary phases in normal-phase liquid adsorption chromatography (high- or low-pressure, LSC).
(1 p.)

(iii) Give 3 examples of physical shapes of silica column packing material that area used in HPLC (Name, and illustrate with drawings).
(2 p.)

4.c) (i) Bonded Phases are available with a variety of different substituents, resulting in a variety of different stationary phase properties. Rank the following popular substituents according to INCREASING polarity (decreasing RP-LC-retention):



alkylphenyl, nitrile (=cyano), diol, octyl, ethyl

(1 p.)

< < < <

Least most polar

(ii) Rank the following solvents according to INCREASING solvent strength for reversed-phase LC (e.g. on a C18 column).

methanol, THF (=tetrahydrofuran), water, dichloromethane, aq. 1M Ammonium sulfate.

(1 p.)

< < < <

Weakest strongest

(iii) Briefly explain what HILIC means (full name and its principle).

(2 p.)

(iv) Which one(s) of the substituents shown in 4.c.(i) can be applied as a column packing material for doing HILIC ?

(1 p.)

4.d) **<u>Either</u>:** What is «*end-capping*» ?
<u>Or :</u> What is a «*brush*» in RP-HPLC ? (Give an alternative name and/or explain.)
<u>Or :</u> What is «*plug elution*» in LC ?

(1 p.)

Question 5 : *(6+5 = 11 p.)*

5.a) (i) What is the lower limit of detection ((l)LOD) ?
(*2 p..)*

(ii) What is the upper limit of ~~detection~~ quantification ?
(1 p.)

(iii) Draw a (hypothetical) calibration curve for Internal Standard calibration (including hypothetical 'raw data'); briefly explain (and name the axes).
(3 p.)

5.b) In the table below some detectors used in chromatography are listed. Fill in the requested information. (Where appropriate, grade using strong – intermediate – weak).
(5 p.)

	Acronym	Applied in GC/LC/CE	Concentration / mass flow sensitive	Universal / selective	Sensitivity (grade)
Flame ionization det.					
UV/-visible light det.					
Electron capture det.					
Thermal conductivity det.					
Refractive index det.					

Question 6 : *(6+4 = 10 p.)*

6.a) (i) Define the fractionation range of a packing material used for size-exclusion chromatography, SEC.

(2 p.)

(ii) Why is gel filtration (aqueous SEC) considered a mild, gentle separation technique, often providing high analyte recoveries. ?

(1 p.)

(iii) What is Blue Dextran used for in analyses by Gel Filtration ?

(1 p.)

(iv) In SEC, two different distribution coefficients are used, K_0 and K_{av} , respectively. Define these two K's, and explain what the difference is between them.

(2 p.)

6.b) (i) Define the term "strongly acidic cation-exchanger", and draw the molecular structure of its "active functional group".

(1 p.)

(ii) Present examples (> 2) of functional (ionogenic / ionic) groups used in "weakly basic anion-exchangers.

(1 p.)

(iii) List two different ways to increase the mobile phase eluent strength in ion-exchange chromatography on a strongly acidic cation-exchanger (keeping the pH constant).

(2 p.)

Question 7 : *(6 p.)*

7.a) (i) Describe (and illustrate with a figure) the schematic layout of a capillary electrophoresis system.

(3 p.)

(ii) In Capillary Electrophoresis all analytes are detected in the detector region near the exit end of the capillary. How are (usually) all analytes moved in the electric field past the detector, irrespective of their charge ?

(2 p.)

(iii) What is the main contributor to band broadening (plate height) in (capillary) zone electrophoresis (CZE) ?

(1 p.)

Question 8 :. *(24 ~~25~~ p.)*

Answer yes/no, and add a (compulsory) brief explanation ("…, because …") :

(1 point pr. correct and correctly explained answer : without explanation a yes/no answer is considered guessing and is valued as follows *: correct answer is + 0,2 points, wrong answer is -0,2 points (subtraction!). For correct yes/no answer with wrong explanation the score is reduced dependent on the explanation.)*

		Yes/No	…, because ….
1.	Maintaining otherwise identical conditions, cellulose-TLC allows better separations than paper chromatography.		
2.	Solubility properties of super-critical fluids are comparable those of gases?		
3.	Mobile phases SFC must be pumped in their liquid state because the supercritical fluid is too compressible.		
4.	Solid-Phase extraction (SPE) is performed in large LC columns using gas-overpressure to achieve fast elution. .		
5.	Solid-Phase extraction (SPE) is economical because a SPE column can be re-used many times.		
6.	The solubility of analytes in supercritical fluids increases as the pressure increases (all other parameters the same).		
7.	In Hydrophilic interaction chroma-tography (HILIC) retention increases with increasing water content of the eluent.		
8.	Bio-affinity chro. (BAC) is normal-phase LC, where the 'ligand' with a strong affinity for the analyte is added to the MP to get separation.		
9.	Silica-based RP columns tolerate much better alkaline conditions than RP columns made from organic polymer particles.		
10.	Hydrophobic interaction chromato-graphy (HIC) is mainly used for analyses of proteins and peptides.		
11.	Solid-Phase extraction (SPE) strongly reduces use of solvents and time when compared to classical liquid-liquid extraction.		
12.	Solid-Phase extraction (SPE) achieves separation using step-wise mobile phase gradients.		
			continued on next page

(Question 8 continued)

Answer yes/no, and add a (compulsory) brief explanation ("because …") :

(1 point pr. correct and correctly explained answer : without explanation a yes/no answer is considered guessing and is valued as follows : correct answer is + 0,2 points, wrong answer is -0,2 points (subtraction!). For correct yes/no answer with wrong explanation the score is reduced dependent on the explanation.)

		Yes/No	…, because ….
13.	Band-broadening by Longitudinal diffusion in gas-solid chromatography (GSC) increases with increasing carrier gas velocity.		
14.	In order to separate enantiomers, either the column (SP) or the eluent (MP) in the chromatographic system must be chiral.		
15.	Eddy-diffusion is a significant contributor to band broadening in CZE.		
16.	Aromatic solvents are especially advantageous for use in HPLC with fluorescence detection.		
17.	The suppressor in a typical ion chromato-graphy system removes the analyte ions after they have passed the detector.		
18.	Electrolytes used in electrophoresis must be pH buffers in order to neutralize H^+ and OH^- formed in the electrolysis at the electrodes.		
19.	In gel electrophoresis analytes are separated according to size, therefore they need not be charged/ionic.		
20.	Reagent added for post-column derivati-sation must be invisible in the detector to allow detection of the derivatized analyte.		
21.	It is not possible to us e the External Standard calibration method when the identity of the analyte is not known.		
22.	The analyses of blank samples is more important for assuring the analysis' accuracy than for its precision.		
23	Using capillary GC columns in a coupled GC/MS, a separator is used to remove the carrier gas, which would disturb the vacuum in the mass spectrometer.		
24	The most common ionization technique in coupled GC/MS is Electron Ionisation (EI).		
25	Modern LC/MS systems make use of MS/MS techniques, because there is no fragmentation with Electrospray Ionisaton (ESI).		