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| **Title** of the subject: **Separation techniques MTMEL7003A** | **ECTS Credit Points: 5** |
| **Type** of the subject: **compulsory** / optional | |
| **Ratio of theory and practice: 50/50** (credit%) | |
| **Type and number of classes per semester**: 28 hour(s) lecture and 28 hour(s) practice per **semester**  Number of teaching hours / week : eg.:2+2 (lecture and practice) | |
| **Type of exam**: **exam** / practical course mark | |
| **Subject in the curriculum:** semester 1 | |
| Preliminary requirements:- | |

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| **Summary of content - theory**: |
| Course objectives:  1. Introduction. Classifications of analytical methods. Performance characteristics of the analytical methods. Selectivity, specificity. Robustness/ruggedness. Range of measurement. Linearity. Sensitivity. Detection limit. Quantitation limit. Accuracy. Precision, repeatability, reproducibility.  2. Chromatography. History of the chromatography. Extraction during chromatography. Chromatography. Terminology of the chromatography. Gas chromatography. Liquid chromatography. Paper chromatography. Thin-layer chromatography. General procedures of the thin layer chromatography. Factors affecting thin-layer separations. Column liquid chromatography. Supercritical fluid chromatography.  3. Physicochemical principles of chromatographic separation. Adsorption (liquid-solid) chromatography. Partition (liquid-liquid) chromatography. Coated supports. Bonded supports. Ion exchange chromatography. Size-exclusion chromatography. Affinity chromatography. Analysis of chromatographic peaks. Chromatographic resolution. Qualitative analysis. Quantitative analysis. Summary of first part of chromatography.  4. The most frequently used chromatographic methods in the practice. High-performance liquid chromatography. Components of an HPLC system. Pumps. Injector. Column. Column hardware. HPLC column packing materials. Detector. Data stations systems. Normal phase HPLC. Stationary and mobile phases. Applications of normal-phase HPLC.  5. Reversed phase HPLC. Stationary and mobile phases. Applications of reversed-phase HPLC. Ion exchange chromatography. Stationary and mobile phases. Ion chromatography. Ion exchange chromatography of carbohydrates and proteins. Size-exclusion chromatography. Column packings and mobile phases. Applications of high performance SEC. Affinity chromatography. Summary of HPLC.  5. Gas chromatography. Sample preparation for gas chromatography, Isolation of solutes from food. Sample derivatization. Gas chromatographic hardware and columns. Gas supply system. Injection port. Oven. Column and stationary phases. Detectors.  6. Chromatographic theory. Separation efficiency. Applications of GC. Residual volatiles in packaging materials. Separation of stereoisomers. Headspace analysis of ethylene oxide in spices. Aroma analysis of heated butter. Total fat by GC for nutrition labelling. Summary of GC.  7. Specific analysis of mono- and oligosaccharides. High-performance liquid chromatography. Stationary phases. Detectors. Gas chromatography. Neutral sugars reduction to alditols. Hydrolyzates of polysaccharides containing uronic acids. Preparation and chromatography of trimethylsilyl (TMS) derivatives. Thin-layer chromatography.  8. Analysis of vitamins by chromatographic methods. Commonly used regulatory methods for vitamin analysis. Determination of vitamin A by HPLC. Determination of vitamin E (tocopherols, tocotrienols) by HPLC. Determination of the fatty acid composition of the fats. Determination of the fatty acid composition as fatty acid methyl esters.  9. Determination of volatile acids (volatile fatty acids) by gas chromatography. Determination of antioxidants. Determination of the amino acid content by gas chromatography. Determination of cholesterol and phytosterols. Separation of lipid fractions by TLC.  10. Protein separation and determination by chromatographic methods. Separation by adsorption. Ion-exchange chromatography. Affinity chromatography.  11. High-performance liquid chromatography. Separation by size. Size-exclusion chromatography.  12. Separation and determination of the amino acids by ion exchange column chromatography applying postcolumn derivatization. Introduction. Sample preparation. Hydrolysis of the protein. Performic acid oxidation before hydrolysis. Hydrolysis methods for the determination of tryptophan. Recent developments in the hydrolysis of the proteins.  13. Ion exchange chromatography of amino acids. Ion exchange resins. Buffer systems for separation of the amino acids. Recent developments in the chromatographic separation. Detection systems. The reaction of the amino acids with ninhydrin. The reaction of the amino acids with other reagents. Controlling of the apparatus and evaluation of the chromatograms. Summary of amino acid analysis.  14. Determination of the amino acids by precolumn derivatization with HPLC. Determination of the protein building amino acids by precolumn derivatization. Determination of D- and L-amino acids by high performance liquid chromatography. Mycotoxin analysis. Detection and determination of mycotoxins. Quantitative and confirmative chemical methods. |
| **Summary of content - practice**: |
| Skills to be learnt:  Tasks and solutions:  Practice 1.  1. Introduction to separation techniques  2. Adopting a new analytical method  3. Evaluation of analytical data  4. Determine the precision of the new method and compare it to the old method  5. Precision, accuracy and specificity of a method  6. Absolute error, relative error  7. Sensitivity and detection limit  8. Correlation coefficient, coefficient of determination  Practice 2.  9. Sampling and Sample Preparation  10. Equipments for collecting a representative sample for analysis  11. Sample bias, change in composition, metal and microbial contamination  Practice 3.  12. Protein Analysis  13. The steps of the Kjeldahl method  14. The conversion factor from Kjeldahl nitrogen  15. Nesslerization  16. Different techniques for the determination of the protein content  Practice 4.  17. Basic principles of chromatography; adsorption, partition, normal phase, reversed phase, cation and anion exchanger, external and internal standards, thin layer and column-liquid chromatography  18. Bonded support, coated support  19. Anion exchange column chromatography of the proteins  20. Size exclusion chromatography (SEC) for determination of the molecular mass of proteins  Practice 5.  21. Stationary phases for protein separation  22. The principle of affinity chromatography; spacer arm  23. Isocratic and gradient elution  24. Quantitate sample components  25. Internal standard, external standard  26. Differences between SFC and LC  Practice 6.  27. High performance liquid chromatography  28. Guard columns  29. Requirements of HPLC column packing materials  30. HPLC detectors  31. Stationary phase with a polar, nonionic functional group  32. HPLC analysis using a column packed with silica gel  33. HPLC and external standards  34. Ion chromatography in food analysis  35. Ion exchange and size exclusion chromatography  Practice 7.  36. Gas Chromatography  37. Solid phase extraction  38. Derivatization before GC analysis  39. Temperature of the injection port at GC analysis  40. Physical characteristics of packed and capillary columns  41. Rises of the baseline  Practice 8.  42. Differentiation between the GC detectors  43. Different separation methods for GC  44. Connection between efficiency and capacity  45. Using internal standard in GC  46. Compare the HPLC and GC chromatographic techniques for separation and determination of different food components  Practice 9.  47. Mass Spectrometry  48. Unique data an MS provide  49. EI and CI ionization  50. Base peak, molecular ion peak at MS  51. Major ions in the in the EI mass spectrum  52. Major differences between the different mass analysers  Practice 10.  53. Analysis of pesticide, mycotoxin, and drug residues in foods  54. Analytical methods provide only estimates  55. Multiresidue, single-residue, and screening methods  56. Five major steps in pesticide analysis  57. Pesticide, mycotoxin and drug residue analysis  58. Immunoassay based analytical methods  59. Microbiological assays for determination of mycotoxin contamination?  60. Sampling procedures for different analyses  61. Screening procedures for mycotoxin, pesticide and drug analysis  62. Mycotoxin analysis by mini-column, commercial kits and HPLC  Practice 11.  63. Vitamin Analysis  64. Extract the vitamins from foods  65. Microorganisms for quantitate vitamins  66. Niacin and folate determination  67. Fluorometric and titrimetric methods for vitamin C content determination  68. Vitamin C forms determination  69. Using HPLC for vitamin analysis  Practice 12.  70. Protein separation and characterization procedures  71. Separation of four different proteins from others  72. Compare the principles of SDS-PAGE and IEF  73. Differences between capillary electrophoresis and SDS-PAGE  Practice 13.  74. Characteristics of the proteins of interest  75. Determination of the amino acid composition of a soy protein  76. Cation exchange column chromatography for separation of amino acids  77. The amino acid profiles of protein supplements sold to body builders  78. Protein quality assay methods  79. Differences between protein quality assay procedures  80. Determine the protein quality of a snack food under various processing  Practice 14.  81. Carbohydrate Analysis  82. Determination of the sugars by GC  83. HPLC vs. GC for carbohydrate analysis  84. RI and PAD detectors in carbohydrate analysis  85. Separation of cellulose, water soluble gums and starch |
| **Literature, handbooks in English** |
| 1. Kovács B – Csapó J.: Modern methods of food analysis. University of Debrecen, Faculty of Agricultural and Food Science and Environmental Managemenet. 2015. 1-205.  2. Nollett, L.M.L. – Toldra, F.: Food analysis by HPLC. CRC Press. Taylor & Francis Group. Boca Raton. 2013. 1-1033.  3. Anderson, J.L. – Berthod, A. – Pino Estevez, V. – Stalcup, A.M.: Analytical Separation sciences. Wiley-VCH Verlag GmbH &Co. KGaA. 2015. 1-1929.  4. Mondello. L. (Ed.): Comprehensive chromatography in combination with mass spectrometry. John Wiley & sons. Inc. 2011. 1-491.  5. Cruz, R.M.S. – Khmelinskii, I – Vieira, M.C.: Methods in food analysis. CRC Press, Taylor & Francis Group. Boca Raton. 2014. 1-250. |
| **Competencies gained** *(acc. to the Regulation on training and outcome requirements)* |
| 1. **Knowledge:**  * Familiar with the separation techniques, and the basic law of different analytical chemistry determination processes involved in the manufacture of good quality foods. * Know the factors determining the basic quality of foodstuffs. * Familiar with the English technical language used in separation techniques.  1. **Skills:**  * Capable for performing routine problems in the process of food production and eliminate them. * Recognize the unity of natural sciences, so the problem solving ability increases. * They will be able to assess the risk of food safety in the food chain of raw materials, for safe storage, and for production and preservation of value-added very good quality safe foods.  1. **Attitude:**  * With the acquisition of the learning material of the separation techniques develops in the student the natural sciences thinking and approach. * Scientific thinking and attitude, professional interest.  1. **Autonomy and responsibility:**  * Due to the safe, accurate and thorough knowledge the students become for independent decision on the field of food production, food analysis food safety. |

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| **Responsible lecturer: Prof. Dr. Csapó János** |
| **Other lecturer(s): -** |

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| **Terms of course completion:** |
| Submitting essay |
| **Form of examination:** |
| Submitting essay |
| **Requirement(s) to get signature:** |
| 80% participation in the lectures and in the practice |

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| **Exam questions:** |
| 1. Identify six reasons you might need to determine certain chemical characteristics of a food product as part of a quality management program.  2. Method A to quantitate a particular food component was reported to be more specific and accurate than method B, but method A had lower precision. Explain what this means.  3. You are considering adopting a new analytical method in your lab to measure the moisture content of cereal products. How would you determine the precision of the new method and compare it to the old method? Include any equations to be used for any needed calculations.  4. Identify a piece of equipment that would be useful in collecting a representative sample for analysis. Describe precautions to be taken to ensure a representative sample is taken and a suitable food product that could be sampled with this device, (b) Identify a piece of equipment that would be useful for preparing a sample for analysis. What precautions should be taken to ensure the sample composition is not changed during sample preparation?  5. The instructions you are following for cereal protein analysis specify grinding a cereal sample to 10 mesh before you remove protein by a series of solvent extractions.  6. Identify five factors that one would need to consider when choosing a moisture analysis method for a specific food product.  7. Why is standardized methodology needed for moisture determinations?  8. What are the potential advantages of using a vacuum oven rather than a forced draft oven for moisture content determination?  9. The procedure for an analysis for moisture in a liquid food product requires the addition of 1–2 ml of deionized water to the weighed sample in the moisture pan. Why should you add moisture to an analysis in which moisture is being determined?  10. A new instrument based on infrared principles has been received in your laboratory to be used in moisture analysis. Briefly describe the way you would ascertain if the new instrument would meet your satisfaction and company standards.  11. A technician you supervise is to determine the moisture content of a food product by the Karl Fischer method. Your technician wants to know what is this "Karl Fischer reagent water equivalence" that is used in the equation to calculate percentage of water in the sample, why it is necessary, and how is it determined. Give the technician your answer.  12. Identify four potential sources of error in the preparation of samples for ash analysis, and describe a way to overcome each.  13. You are determining the total ash content of a product using the dry ashing method. Your boss asks you to switch to a wet ashing method because he/she has heard it takes less time than dry ashing.  a. Do you agree or disagree with your boss concerning the time issue, and why?  b. Not considering the time issues, why might you want to continue using dry ashing, and why might you change to wet ashing?  14. Your lab technician was to determine the ash content of buttermilk by conventional dry ashing method. The technician weighed 5 g of buttermilk into one weighed platinum crucible, immediately put the crucible into the muffle furnace using a pair of all stainless steel tongs, and ashed the sample for 48 hr at 800 °C. The crucible was removed from the muffle furnace and set on a rack in the open until it was cool enough to reweigh. Itemize the instructions you should have given your technician before beginning, so there would not have been the mistakes made as described above.  15. Identify an advantage and disadvantage of using microwave wet digesters or microwave muffle furnaces compared to conventional units.  16. What are some important considerations when selecting solvents to be used in continuous and non-continuous solvent extraction methods?  17. To extract the fat from a food sample, you have the choice of using ethyl ether or petroleum ether as the solvent, and you can use either a Soxhlet or a Goldfish apparatus. What combination of solvent and extraction would you choose? Give all the reasons for your choice.  18. Itemize the procedures that may be required to prepare a food sample for accurate fat determination by a solvent extraction method (e.g., Soxhlet method). Explain why each of these procedures may be necessary.  19. You performed fat analysis on a new super-energy shake (high carbohydrate and protein) using standard Soxhlet extraction. The value obtained for fat content was much lower than expected. What could have cause d the measured fat content to be low and how would you modify the standard procedure to correct the problem?  20. Which of the following methods are volumetric and which are gravimetric determinations of lipid content: Soxhlet, Gerber?  21. What factors should one consider when choosing a method for protein determination?  22. The Kjeldahl method of protein analysis consists of three major steps. List these steps in the order they are done, and describe in words what occurs in each step. Make it clear why milliliters of HC1 can be used as an indirect measure of the protein content of a sample.  23. Why is the conversion factor from Kjeldahl nitrogen to protein different for various foods and how is the factor of 6.25 obtained?  24. How can Nesslerization or the procedure that uses phenol and hypochlorite be used as part of the Kjeldahl procedure, and why might they best be put to use?  25. Give three reasons why carbohydrate analysis is important.  25. "Proximate composition" refers to analysis for moisture, ash, fat, protein, and carbohydrate. Identify which of these components of "proximate composition" are actually required on a nutrition label. Also, explain why it is important to measure the nonrequired components quantitatively if one is developing a nutrition label.  26. Distinguish chemically between monosaccharides, oligosaccharides, and polysaccharides, and explain how solubility characteristics can be used in an extraction procedure to separate monosaccharides and oligosaccharides from polysaccharides.  27. Discuss why mono- and oligosaccharides are extracted with 80% ethanol rather than with water. What is the principle involved?  28. What are the principles behind total carbohydrate determination using the phenol-sulfuric acid method? Give an example of another assay procedure based on the same principle.  29. What is the principle behind determination of total reducing sugars using the Somogyi-Nelson and similar methods?  30. Describe the principle behind anion-exchange chromatography of carbohydrates.  31. Describe the general procedure for preparation of sugars for GC. What is required for this method to be successful?  32. Why has HPLC largely replaced GC for analysis of carbohydrates?  33. What is the advantage of an enzymatic method? What is the limitation (potential problem)?  34. Describe the principles behind the enzymatic determination of starch. What are the advantages of this method? What are potential problems?  35. Describe the principles behind separation and analysis of cellulose, water-soluble gums, and starch.  36. List the constituents of dietary fibre.  37. What factors should be considered in selecting the assay for a particular vitamin?  38. To be quantitated by most methods, vitamins must be extracted from foods. What treatments are commonly used to extract the vitamins? For one fat-soluble vitamin and one water-soluble vitamin, give an appropriate extraction procedure.  39. Explain why it is possible to use microorganisms to quantitate a particular vitamin in a food product, and describe such a procedure.  40. Niacin and folate both can be quantitated by microbiological methods. What extra procedures and precautions are necessary in the folate assay compared to the niacin assay, and why?  41. During processing and storage of foods, L-ascorbic acid can be oxidized to L-dehydroascorbic acid. Using the 2,6-dichloroindophenol titrimetric method for vitamin C, how could you quantitate total vitamin C and each form individually?  42. What are the advantages and disadvantages of using HPLC for vitamin analysis?  43. What is the major concern in sample preparation for specific mineral analysis? How can this concern be addressed?  44. If the ammonia buffer is pH 11.5 rather than pH 10 in the EDTA complexometric titration to determine the hardness of water, would you expect to overestimate or underestimate the hardness? Explain your answer.  45. You have decided to purchase an ISE to monitor the sodium content of foods produced by your plant. List the advantages this would have over the Mohr/Volhard titration method. List the problems and disadvantages of ISE that you should anticipate.  46. What factors should be considered in selecting a specific method for mineral analysis for a food product?  47. How would you recommend determining the endpoint in the titration of tomato juice to determine the titratable acid? Why?  48. Why is volatile acidity useful as a measure of quality for acetic acid fermentation products, and how is it determined?  49. Could a sample that is determined to contain 1.5 percent acetic acid also be described as containing 1.5 percent citric acid? Why or why not?  50. Peroxide value, TBA number, and hexanal content all can be used to help characterize a fat sample. What is the definition of peroxide value, TBA number and hexanal content?  51. What methods would be useful in determining the effectiveness of various antioxidants added to an oil?  52. The Nutrition Education and Labelling Act of 1990 requires that the nutrition label on food products contains information related to lipid constituents. In addition to the amount of total fat, the label must state the amount of saturated fat and the cholesterol content. For a product such as traditional potato chips, explain an appropriate method for the analysis of each of these lipid constituents  53. Explain how capillary electrophoresis differs from SDS-PAGE.  54. Explain, on a chemical basis, why extremes of pH and temperature can reduce the rate of enzyme-catalysed reactions.  55. You believe that the food product you are working with contains a specific enzyme inhibitor. Explain how you would quantitate the amount of enzyme inhibitor (I) present in an extract of the food.  56. What methods can be used to quantitate enzyme activity in enzyme-catalysed reactions?  57. All immunoassays have two conditions that they must satisfy; what are they?  58. Describe, in general terms, how you would use immunoaffinity purification to isolate a protein for which you have developed antibodies.  59. Give four common applications of immunoassays in food analysis.  60. What is meant by the "tolerance level" for a pesticide on a fresh agricultural product?  61. Why haven't microbiological assays been developed to detect the presence of toxicogenic fungi in fresh or stored produce and used as an indicator of possible mycotoxin contamination of that product?  62. Which phenomena associated with light are most readily explained by considering the wave nature of light? Explain these phenomena based on your understanding of interference.  63. What does it mean to say that the energy content of matter is quantized?  64. Molecular absorption of radiation in the UV-VIS range results in transitions between what types of energy levels?  65. Why is it common to use absorbance values rather than transmittance values when doing quantitative UV-Vis spectroscopy?  66. Considering a typical spectrophotometer, what is the effect of decreasing the exit slit width of the monochromator on the light incident to the sample?  67. What would be the advantages of having an atomic absorption unit that had a graphite furnace?  68. You are performing iron analysis on a milk sample using AAS. Your results for the blank are high. What could be causing this problem and what is a possible remedy?  69. What are the basic components of an MS?  70. What are the unique aspects of data that an MS provides? How is this useful in the analysis of foods?  71. What is the advantage of bonded supports over coated supports for partition chromatography?  72. Would you use a polystyrene- or a polysaccharide-based stationary phase for work with proteins? Explain your answer.  73. What is gradient elution from a column, and why is it often advantageous over isocratic elution?  74. You are performing HPLC using a stationary phase that contains a polar nonionic functional group. What type of chromatography is this, and what could you do to increase the retention time of an analyte?  75. Why must sugars and fatty acids be derivatized before GC analysis, while pesticides and aroma compounds need not be derivatized?  76. Differential centrifugation of the liver particles for determination of the different organelles.  77. Isopycnic (sucrose density) centrifugation for determination of the different cell particles.  78. Absorption of ultraviolet light by aromatic amino acids, and its used in analytical chemistry.  79. Column chromatography for the purification of the different protein molecules.  80. The use of ion exchange, size-exclusion and affinity chromatography for the separation and purification of the proteins.  81. Electrophoresis for separation and characterization of the different proteins.  82. SDS (sodium dodecyl sulphate) poly acryl amid electrophoresis for the determination of the molecular mass of different proteins.  83. Isoelectric focusing for separation of proteins according to their isoelectric points.  84. Two dimensional electrophoresis.  85. Common procedures in the extraction, separation, and identification of cellular lipids.  86. Adsorption chromatography for separation lipids of different polarity.  87. Using of reduced (NADPH) and oxidised (NAD) coenzymes for the determination of different substrates.  88. Determination of the amino acid composition of different protein-containing materials. Sample preparation: Hydrolysis of the protein, acidic hydrolysis.  89. Determination of the amino acid composition of different protein-containing materials. Sample preparation: Performic acid oxidation before hydrolysis for the determination of the sulphur containing amino acids.  90. Determination of the amino acid composition of different protein-containing materials. Sample preparation: Hydrolysis methods for the determination of tryptophan.  91. Determination of the amino acid composition of different protein-containing materials. Sample preparation: Recent developments in the hydrolysis of the proteins.  92. Ion-exchange chromatography of amino acids: Ion-exchange resins.  93. Ion-exchange chromatography of amino acids: Buffer systems for separation of the amino acids, choice of buffer system, effect on separation by pH, temperature, organic solvents and column flow rate.  94. Ion-exchange chromatography of amino acids: Preparation of the sodium citrate buffers, preparation of the lithium citrate buffers, recent developments in the chromatographic separation.  95. Ion-exchange chromatography of amino acids: Detection systems. The reaction of the amino acids with ninhydrin. Preparation of the ninhydrin reagent.  96. Ion-exchange chromatography of amino acids: The reaction of the amino acids with other reagents.  97. Ion-exchange chromatography of amino acids: Controlling of the apparatus and evaluation of the chromatograms.  98. Ion-exchange chromatography of amino acids: Determination of the free amino acid content of biological materials.  99. Determination of the heat sensitive antinutritive factors in fullfat soybean  100. Separation of the D-amino acids with high performance liquid chromatography.  101. Separation and determination of the different proteins with polyacrylamide gel electroforesis and isoelectric focusing.  102. Determination of the molecular mass of proteins with SDC (sodium dodecyl sulphate) PAGE. |