

# **ФАРМАЦЕВТИЧЕН ФАКУЛТЕТ**МЕДИЦИНСКИ УНИВЕРСИТЕТ-СОФИЯ

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### **Approved from the Faculty council with Protocol** № 4/23.04.2015

**DEAN:** 

(prof. Al. Zlatkov, DSc)

#### DEPARTMENT OF PHARMACOGNOSY

#### **SYLLABUS**

of

#### **ELECTIVE DISCIPLINE**

## "METHODS FOR THE ANALYSIS OF SECONDARY METABOLITES IN PLANT PRODUCTS"

INCLUDED IN "PHARMACY" EDUCATION CURRICULUM.

DEGREE OF EDUCATION: "MASTER"

CREDITS (ECTS): 5

#### **ANNOTATION**

In the last decade, important initiatives have been started by AOAC (Association of analytical communities) in collaboration with NIH (National institutes of health) and FDA (Food and drug administration) to develop methods for the control of plant products, with the aim of monitoring their quality and safety. It is necessary to select suitable markers, develop inter-laboratory validated (including chromatographic) methods and international standardization procedures that will be used by regulatory agencies and laboratories. For each product of plant origin, whether it is a food, nutritional supplement, medicinal or cosmetic product, the quality of the plant substances and final products is of great importance to the consumer.

Corresponding to the high requirements imposed by the international and local institutions, the competences of future specialists in pharmacy can be expanded in the direction of learning theoretical and practical knowledge related to modern methods in phytochemistry and their application for the control of products with plant origin. Considering as a prerequisite the large number of innovative technologies for the preparation and analysis of bioactive substances (BAS) and the global trend to increase the intake of drugs and nutritional supplements with plant origin, the Department of Pharmacognosy performs the elective discipline "Methods for the Analysis of Secondary Metabolites in Plant Products".

**Aim:** The discipline aims to acquaint students with the theoretical details of the methods for the analysis of secondary metabolites of plant origin, as well as their application in the plant

products research and monitoring, as well as widely used medicinal plants in pharmaceutical practice. The **lectures** aimed at practical aspects of the methods for the analysis of the main secondary metabolites classes from plant matrices. Emphasis will be placed on the new approaches in sample preparation and combined chromatographic techniques (liquid chromatography-mass spectrometry, gas chromatography-mass spectrometry, liquid chromatography - nuclear magnetic resonance). An essential part of the lectures and practicals s is aimed at the application of liquid chromatographic methods for the analysis of widely used medicinal species and products with plant origin.

In the **laboratory exercises**, students will learn basic instrumental methods used in the study of different secondary metabolites in plants and acquire practical skills in the application and development of methods for the analysis of plant extracts and nutritional supplements.

The expectations of the offered curriculum are aimed at the creating of competencies in the specialist of pharmacy to find realization in the field of phytochemistry, the analysis of products with plant origin, plant substances and phytoproducts.

Type of control and evaluation: 2 (two) seminars and an exam at the end of the semester.

### **English language training**

#### **SILLABUS**

#### Spectral methods for the analysis of plant secondary metabolites

- 1. Structural analysis by IR and UV absorption spectra. Application of spectral methods for the identification of secondary metabolites with plant origin.
- 2. Application of mass spectrometry in the identification of main classes secondary metabolites.
- 3. Application of nuclear magnetic resonance spectroscopy for the identification of glycosides: identification of aglycones and sugars by one- and two-dimensional NMR techniques.
- 4. Application of nuclear magnetic resonance spectroscopy for identification of alkaloids by oneand two-dimensional NMR techniques.

# Хроматографски методи в анализа на вторични метаболити от растителен произход Chromatographic methods in the plant secondary metabolites analysis

- 5. Sample preparation methods. Types of plant matrices. Liquid extraction, liquid-liquid extraction, soxhlet, solid-phase extraction, solid-phase microextraction, solid-phase matrix dispersion. Vapor phase solid phase extraction.
- 6. Liquid-chromatographic methods in the analysis of plant secondary metabolites. Development of methods for high performance liquid chromatography (HPLC). Approaches in the selection of sorbents, mobile phases and detection in HPLC. Potential and limitations of using refractometric, ultraviolet, fluorescent, electrochemical detectors and diode array detection.
- 7. Analysis of non-ionized and ionized analytes HPLC on sorbents with normal and reversed phases, ion exchange HPLC and HPLC with ion pairs. Semi-preparative high-performance liquid chromatography.
- 8. Hyphenated HPLC methods. Liquid chromatography mass spectrometry (LC-MS) method

- for identification and quantification of plant secondary metabolites. Thermospray, electrospray and chemical ionization at atmospheric pressure interfaces in hyphenated liquid-chromatographic techniques.
- 9. Hyphenated liquid-chromatographic methods. Liquid Chromatography Nuclear Magnetic Resonance (LC-NMR), Liquid Chromatography Photodiode Array Nuclear Magnetic Resonance (LC-DAD-NMR), Liquid Chromatography Nuclear Magnetic Resonance/Mass Spectrometry (LC-NMR/MS).
- 10. Application of gas chromatography-mass spectrometry (GC-MS) method for the separation and quantification of secondary metabolites.
- 11. Evaluation of the chromatographic results. Method validation. Uncertainty of chromatographic methods. Application of the chemometric approach in with chromatographic and spectroscopic methods for quantitative indicators processing.

#### Application of HPLC in the analysis of secondary metabolites in plant products

- 12. Application of HPLC in the analysis of secondary metabolites of the shikimic acid biosynthetic pathway: phenolic acids, tannins, coumarins, flavonoids, lignans, etc.
- 13. Application of HPLC in the analysis of secondary metabolites from the isoprene biosynthetic pathway: monoterpenes, sesquiterpenes, diterpenes, triterpenes (including saponins), carotenoids, sterols, iridoids.
- 14. Application of HPLC in the analysis of secondary metabolites- amino acids derivatives: indole, isoquinoline, tropane alkaloids, etc.
- 15. Uses of "fingerprints" and markers in the plant substances identification. HPLC analysis of plant secondary metabolites in medicinal products and food supplements from *Ginkgo*, *Tribulus*, milk thistle, Echinacea, ginseng, hawthorn, aloe.

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