

## HABILITATION THESIS REVIEWER'S REPORT

### Masaryk University

<b>Applicant</b>	Mgr. Danuše Tarkowská, Ph.D.
<b>Habilitation thesis</b>	Trace and ultra-trace analysis of natural substances of terpenoid character
<b>Reviewer</b>	RNDr. Václav Kašíčka, CSc.
<b>Reviewer's home unit, institution</b>	Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Praha ...

The submitted habilitation thesis of Mgr. Danuše Tarkowská, Ph.D., summarizes results of her scientific research activity in the period 2013-2023. It represents a comprehensive set of altogether 16 scientific publications – three review articles and 13 research articles. The common theme of them is the development of highly efficient separation methods, high and ultra-high performance liquid chromatographic techniques (HPLC/UHPLC) hyphenated with highly sensitive tandem mass spectrometry (MS/MS) detection and their application for trace and ultra-trace analyses of important biologically active compounds – natural substances of the plant origin. The analyzed compounds include various terpenoid-like compounds, mainly two classes of plant hormones, gibberellins and brassinosteroids, and other natural substances of terpenoid character, phytoecdysteroids and indole alkaloids.

The topic of the thesis is very actual and important. The development of new highly efficient and highly sensitive analytical methods is extremely important since the results of analysis of complex mixtures of natural biologically active compounds in plants can contribute to explanation of molecular bases of biological processes in plants and to the new discoveries in plant biology and physiology. The new methods can reveal new ways of transformation of biologically active compounds and they can help to find new compounds with various biological activities that can become bases for new drugs, pesticides, insecticides, growth factors and other useful substances.

The aim of the thesis was to contribute to the development of new methods and to elaborate new procedures for qualitative and quantitative analysis of natural plant compounds, covering a wide spectrum of compounds of terpenoid character in complex biological matrices, extracts of different plants and different plant organs. This aim were successfully achieved. New methods have been developed and applied in the study of occurrence, biosynthesis, metabolism and properties of the above low-abundance various types of natural compounds of terpenoid character.

The papers were mostly published in prestigious international peer-reviewed journals with relatively high impact factors, both in the area of plant science, such as *Planta*, *Nature Plants*, *Plant Physiology*, *Environmental and Experimental Botany*, and in the analytical chemistry journals, such as *Talanta* and *Analytical and Bioanalytical Chemistry*. The significant contribution of Dr. Tarkowská to these papers is confirmed by the fact that in a half of them she is the corresponding and/or the first author and in the other papers, she is mostly the second or third author.

The general evaluation of the thesis is positive. However, to fulfill the role of a reviewer I have a few critical comments.

1. The numbering of the sections using a combination of Roman and Arabic numerals is not "the best one"; the deeper numbering of sections is missing and the structure of thesis is

thus not quite clear. The content of the thesis is presented too briefly, only the titles of the main sections are presented and the titles of many subsections and sub-subsections are missing.

2. The list of abbreviations is incomplete. Many of them are missing and some of them are not explained when they are first used in the text.
3. In some cases, the terminology is not correct. The obsolete term "molecular weight" and the abbreviation "MW" should be replaced for the official term "relative molecular mass" and the symbol " $M_r$ ", respectively. In principle incorrect is to present MW (dimensionless quantity) using the unit "g/mol", which is the unit another quantity, molar mass. The term "dissociation constant" should be replaced for the term "acid dissociation constant" or "acidity constant". When the sample amount is expressed in grams (= unit of mass), the term "weight" should be replaced for the term "mass". The systematic name of isoprene is 2-methylbuta-1,3-diene, not 2-methyl-1,3-butadiene. In addition, all percent concentrations should be specified as % m/m or % m/v or % v/v.

### Reviewer's questions for the habilitation thesis defence (number of questions up to the reviewer)

1. In the legend of Fig. 4, page 19, the substance amount of analyzed 20 gibberellins is presented as 10 pmol. What was the injection volume and thus, what was the concentration of the analyzed compounds in the presented analysis and in the other "typical analyses" of real biological samples.
2. The number of "to date" identified brassinosteroids is presented as "more than 70" (page 26, top) and the number of phytoecdysteroids is presented as "around 300 in more than 100 species" (page 40, bottom). However, the references for these numbers, ref. [59] for the former number and ref. [103] for the latter numbers, are from the years 2007 and 2002, respectively. What are the current estimates of the number of identified brassinosteroids and phytoecdysteroids?
3. Are there any advances in the strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS than those presented in ref. [181] from 2003?
4. How do you see the potential of capillary electromigration methods, especially the electrokinetic chromatography and electrochromatography (online coupled with tandem MS detection) that are able to separate neutral compounds, in the analysis of terpenoid-like compounds?
5. Where do you see additional reserves for increasing i) sensitivity and ii) selectivity of the currently used analytical methods? In the pre-separation and separation methods or in the detection techniques?

### Conclusion

The review and research articles of Dr. Danuše Tarkowská, collectively presented in the submitted habilitation thesis, represent a significant contribution to the development of new high-efficient and high-sensitive analytical methods and their application for preconcentration, separation, quantification and characterization of natural substances of plant origin. I recommend to accept the submitted thesis as a basis for awarding Dr. Danuše Tarkowská with the scientific-pedagogical title "docent".

The habilitation thesis entitled "Trace and ultra-trace analysis of natural substances of terpenoid character" by Danuše Tarkowská fulfils – **does not fulfil** requirements expected of a habilitation thesis in the field of Analytical Biochemistry.

Date: 27.12.2023

Signature: Václav Kašíčka, v.r.