

**CHARLES UNIVERSITY IN PRAGUE**  
**FACULTY OF PHARMACY IN HRADEC KRALOVE**  
Department of Biochemical Sciences

Pharmacy

**Review of diploma thesis**

Student: **Matthildi Kanavi**

Mentor of the thesis: PharmDr. Petra Malátková, Ph.D,

Reviewer of the thesis: PharmDr. Hana Štambergová,  
Ph.D.

Year of the defence:  
2015

Title of diploma thesis:

**In vitro biotransformation study of fenofibric acid**

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Formal comments: number of pages: 70, number of graphs: -, number of figures: 14  
(including graphs),

number of tables: 16, number of references: 85

Type of work: Experimental

- a) Aim of the thesis is: Fulfilled
- b) Language and graphic level: Good
- c) Processing of theory: Very good
- d) Methods description: Very good
- e) Results description: Good
- f) Discussion and conclusions: Good

Reviewer comments:

The submitted diploma thesis is focused on determination of involvement of particular cellular fractions/enzymes in biotransformation of commonly used hypolipodemic agent fenofibric acid. This process has not been fully understood so far, and in this respect, the outputs of the current study are noteworthy. The theoretical part (22 pages) is processed quite thoroughly, with just minority typing or grammatical errors and inaccuracies (e.g. page 14 - NAD(H) vs NADP(H) or page 24 - 46,000 SDR members). I would disagree with the statement that AKR superfamily with 15 members represents the biggest group of NAD(P)(H) dependent oxidoreductases in human (compared to at least 75 human SDRs). I would appreciate the structure of fenofibrate as it is mentioned several times as parent compound of fenofibric acid. The chapters 'Materials' and 'Methods' are well arranged, however, there are inconsistencies in writing style (dots vs decimal points, spaces between numbers and units; completeness of information about chemicals and instruments). The expression of protein concentration is used in uncommon manner which makes it little bit confusing (page 37). Several times the abbreviations are used without explanation (e.g. IS, FA; they are missing in the list of abbreviations). In the part 'Results and discussion' the set of graphs as well as results arranged in tables facilitate the orientation in big amount of data. Nevertheless, I would doubt about interpretation of several results. For example in chapter 6.2 there is no significant difference between extraction efficiency of samples C3-1/e and C3-1/nhe considering the standard deviations; similarly on page 54 where is compared the enzymatic activity of samples obtained by Bug Buster vs. sonication approaches. In the chapter 6.2 page 47 ('Optimization of extraction') I missed the reason of determination of specific enzymatic activity with the goal to conclude which extraction conditions would be better. In the chapter 6.3 'Calibration curve' the graph lacks the legends. In the chapter 6.6 'Enzyme

kinetics' it is quite tricky to interpret data obtained for cytosol as every single measurement resulted in different enzyme kinetic.

Questions:

- 1) Could you explain the sorting of particular compounds into the four groups? Table 3, page 18.
- 2) Based on my experience you used high concentration of protein in enzymatic reaction (100 µg of cellular fraction per reaction resp. 20 µg or purified recombinant enzyme per reaction). Why didn't you try lower protein concentration? (Table 12, page 53)
- 3) What is the second peak on the chromatograms – Graph 3 resp. Graph 5, page 43 resp. 44?
- 4) With regard to relatively high tested concentration of fenofibric acid and  $K_m$  value - do you know what is the level of fenofibric acid in human body after administration? Is it possible to achieve the tested concentration of this substrate in the cells?
- 5) How do you explain 3 different results of enzyme kinetics you received for cytosol as for all samples was used the same stock? And what is different between cytosol and pooled cytosol resp. microsomes and pooled microsomes?
- 6) Why didn't you test some microsomal enzymes? As the microsomal fraction seems to be more active.

**Evaluation of diploma thesis: Very good, to defense: Recommended**

In Hradec Kralove 29.5.2015

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