



**Confidential**

### Review of USB FFPW PhD Thesis

<b>First name(s), surname, titles of the PhD student:</b> Ganna Fedorova, M.Sc.	<b>First name(s), surname, titles of supervisor:</b> Mgr. Roman Grabic, Ph.D.
<b>Title of PhD thesis:</b> Fate of polar organic pollutants in aquatic environment	

**REVIEWER:**

<b>Surname:</b> Pacáková	<b>Institution:</b> Univerzita Karlova v Praze Přírodovědecká fakulta Katedra analytické chemie Albertov 2030 128 40 Praha 2
<b>Name:</b> Věra	<b>E-mail:</b> pacakova@natur.cuni.cz
<b>Titles:</b> Prof. RNDr., CSc.	<b>Please describe your field of expertise:</b> Analytical chemistry, Separation science
<b>Please describe your professional relationship to the PhD student:</b> None	

### QUESTIONNAIRE

**Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research**

Evaluate competitiveness of the PhD thesis in the international context and compare its level with the current state of the art in the field (**extent ¼ – ½ page**):

This thesis deals with very interesting and timely topic. Emerging organic pollutants in aquatic environment are receiving considerable attention and their monitoring is of great importance due to their potential adverse effects on aquatic ecosystem. Though a lot of work in this field has been done, sensitive methods for determination of trace levels of these pollutants in complex matrices are still needed. The author achieved important goals: She developed multi-residual methods based on liquid chromatography coupled with triple quadrupole mass spectrometry and with high resolution mass spectrometry for monitoring pollutants in different environmental matrices, i.e., waste water, surface water and fish, with focus on pharmaceuticals, illicit drugs and perfluorinated compounds. The new methods have a number of advantages over the existing ones: they can analyze a number of pollutants in a single run and quantify them at trace levels. Important factors influencing the analytical results, such as sample storage and matrix effects, were studied as well.

The thesis is based on original ideas and meets international standards. Results presented in this thesis are of importance for both basic and applied research. Monitoring of pharmaceuticals and illicit drugs in waste water can be used as indicators of their consumptions. Analysis of biota samples is an essential part in the assessment of



pollutants fate in aquatic environment. The study presented in the thesis include 32 antibiotics from different classes determined in total 97 samples of fish and shrimp and was the first of this kind in the Czech Republic.

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***Elaboration of the PhD thesis, objectives of the work and deliverables***

Evaluate the overall level of elaboration of the PhD thesis (structuring of the main text, comprehensibility, logicity of the chapters and their ordering) and the originality of the selected approaches to solve the objectives; evaluate publications and whether the results described correspond to objectives of the PhD thesis (**extent ¼ – ½ page**):

The thesis is well structured and balanced. The chapters are logically arranged. The thesis is well written and gives a very good example of the importance of the full description and validation of analytical protocols. The approaches to solve the objectives are original.

The thesis is based on 10 original papers: five of them have already been published in international journals, where they passed the rigorous reviewing. One paper is in press and 4 of them were sent to publishers. (Have they been in the meantime accepted for publication?)

The results of publications correspond to the objectives of the PhD thesis.

The thesis also shows that the Ph.D. candidate has a great capability for fruitful collaboration with other institutes, i.e., the Chemistry Department of Umea University, Sweden, in LC/MS/MS method development, which resulted in 3 publications.

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***OVERALL COMMENTARY ON THE PhD THESIS***

**Please write comments in extent of 1-2 pages:**

The thesis is an important contribution to the multi-residual analysis polar organic pollutants in aquatic environment. The thesis is treated as set of five chapters. Their titles give a good picture of the actual contents. Chapter 1 is devoted to literature survey; Chapter 2 is a compilation of 3 papers, dealing with new analytical methods for the monitoring of emerging pollutants (pharmaceuticals, illicit drugs) in aquatic environment. Chapter 3 deals with passive sampling of perfluorinated acids and sulfonates (1 paper), Chapter 4 with the assessment of storage effect of waste water samples on analysis of pharmaceuticals and personal care products and with estimation of Tamiflu use and compliance from measured concentrations in eluent water (results of 2 papers). Discussion and summary are presented in Chapter 5. Finally the author made suggestions concerning further research areas in this field.

Modern multi-residual methods have been developed based on liquid chromatography coupled with triple quadrupole mass spectrometry and with high resolution mass spectrometry. All the methods were fully validated in terms of recovery, repeatability, selectivity and sensitivity (limit of quantification). The new methods have a number of advantages over the existing ones: they can analyze a number of pollutants in a single run and quantify them at trace levels. Important factors influencing the analytical results, such



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in České Budějovice  
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as sample storage and matrix effects, were studied as well.

The aims of the thesis were completely fulfilled. The author has received original analytical results which can be directly applied in praxis in monitoring of polar organic pollutants including pharmaceuticals, illicit drugs and perfluorinated compounds in waste waters, surface water and in fish.

Some remarks: In publication "Simultaneous determination of 32 antibiotics in aquaculture products using LC-MS/MS" units  $\mu\text{g}\cdot\text{kg}^{-1}$  are incorrectly written as  $\mu\text{g}\cdot\text{kg}^{-1}$ .  
The same publication, p. 43: "shrimps of Czech origin" – what it means?  
How did you recognize the origin of fish?

The thesis demonstrates the creativity of the candidate, her ability to tackle difficult analytical problems and suggest original solution. According to my opinion this thesis meets international standards and there is no doubt that PhD. degree can be awarded.

## **FINAL RECOMMENDATION**

- PhD Thesis can be recommended for defence**  
 **PhD Thesis can be recommended with reservations for defence**  
 **PhD Thesis can not be recommended for defence**

Prague June 3, 2013

.....  
Date and place

Věra Pacáková

.....  
Name and signature



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<b>Title of PhD thesis:</b> Fate of polar organic pollutants in aquatic environment	
<b>REVIEWER:</b>	
<b>Surname:</b> Grochowalski	<b>Institution:</b> Cracow University of Technology Warszawska 24 St. 31-155 Kraków Poland
<b>Name:</b> Adam	<b>E-mail:</b> agrochow@chemia.pk.edu.pl
<b>Titles:</b> Dr. hab. Ing,	
<b>Please describe your professional relationship to the PhD student:</b>	<b>Please describe your field of expertise:</b>

**QUESTIONNAIRE**

**Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research**

Evaluate competitiveness of the PhD thesis in the international context and compare its level with the current state of the art in the field (**extent ¼ – ½ page**):

The presented PhD thesis put a new light on the very actual problems concerning the complexity of the determination of polar organic compounds in the Environment. It should be emphasized, that, however, there are many scientific work performed and published in such area - the presented work is novel and gives a big number of data of such compounds as polar pesticides, antibiotics, perfluorinated organic acids and its sulfonates as well as a new pharmaceuticals. In the last group, the antiviral chemical oseltamivir (Tamiflu®) has been widely studied in waste water systems in England as an international activity. It gives quite new information about fate of this particular contaminant in aquatic ecosystem.

The experiments have provided a lot of extremely valuable data which may be useful for the routinely control of polar environmental pollutants using up-to-date analytical instruments. The data obtained for such big number of different polar organic compounds determined using high resolution mass spectrometry as combined quadrupole-orbitrap analyser gave quite new results in sensitivity and selectivity of the method. I can see an important practical aspect of the work giving the powerful tool for modern analytical laboratories determining PPCPs and similar, hazardous polar organic compounds in the aquatic environment. It should be emphasized, that the described methods may be considered as a green chemistry that minimize the use of toxic organic solvents still used for liquid/liquid extraction in many laboratories and so on.

I regard as very valuable the studying of the effect of simultaneously passive sampling and the determination of different kinds of polar organic compounds in one analytical run, using novel (invented by Dr. Roman Grabic), multiresidual analysis method that have never been published so far.

I would like to point out that the experiments have been performed in an international scale. As I good understand



most experiments have origin from Umeå University in Sweden as well as from University of South Bohemia in České Budějovice in Czech Republic.

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### **Elaboration of the PhD thesis, objectives of the work and deliverables**

Evaluate the overall level of elaboration of the PhD thesis (structuring of the main text, comprehensibility, logicity of the chapters and their ordering) and the originality of the selected approaches to solve the objectives; evaluate publications and whether the results described correspond to objectives of the PhD thesis (**extent ¼ – ½ page**):

Elaboration level of the presented PhD thesis is very high. Main text is well organized and presented. All necessary information and data is adequate to the area of the experiment and the result discussion. The statement of objectives of the manuscript is adequate and appropriate in view of the subject matter. All results are well documented. The title of this manuscript clearly reflects its content; the abstract is sufficiently informative. All tables and figures are necessary and complete.

I want to underline, that most of the results from the experimental work are published in scientific publications of international circulation and are presented in this form in the doctoral elaboration.

Simply the document is complete. English language is excellent.

Ganna Fedorova, M.Sc. has studied and cited an appropriate number of scientific publications used and quoted in the thesis. It is the evidence that she is very knowledgeable about the problem discussed in the thesis.

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### **OVERALL COMMENTARY ON THE PhD THESIS**

**Please write comments in extent of 1-2 pages:**

First of all I want to underline that the elaboration with details describes investigated analytical problem, performed experiment and the discussion is, clearly presented and well documented. The work is devoted to very important problem concerning the environmental distribution and fate of hazardous polar organic compounds. The origin of the investigated compounds is anthropogenic. Therefore the knowledge of way of their distribution, bioaccumulation and biomagnification is very important to find a successful method giving a tool for minimizing of releasing POCs into the aquatic environment.

I am wonder why, the only one antibiotic (flumequine) has been quantified if fish tissue samples from Czech market where many of them are commonly used in fish feeding systems in fish farms in Europe. As it is indicated in this elaboration, the European use is more widely in spectrum of specific antibiotics than in USA and Canada. How it can be explain? Do they decompose in the fish



organism or the decomposition takes place in aquatic system?

From the work, I good know that the selectivity and sensitivity of the analytical method is excellent. Therefore, trying to explain these phenomena might be very interesting from scientific reason.

It is noteworthy that one of the strongest sides of the study is the investigation on storage effect with regard on the stability of the target compound (however, only PPCPs), what is widely discussed in this work.

It should be emphasized that in many analytical activities the target compound loss in the sample caused by many chemical/biological and physical processed is not recognized yet or simply not taken into account at all!

Is it possible, that the target compound is decomposed in POCIS sampler during such long sampling period? Its stability in the form as sorbed, however, should be much higher than in the form as dissolved in water but the phenomena is not recognized and not explained yet. It is essential, because it has been shown in this work that in wastewater treatment plants the analyte concentration has to be found in greater concentration in out-coming stream than in in-coming stream.

Appreciating the huge scope of the experimental and analytical work, I cannot make any reservations about the scope of the experiment, but there is one suggestion for future experiments – it might be useful to explore the area of the target compound decomposition pathways.

I would like to point out that the data obtained in the study can be priceless in supplementing and corroborating the factors for the calculation of real concentration of the target compounds in aqueous environment what is practically impossible in passive sampling. Mostly from the reason of many physical/chemical and biological parameters that can vary in the real environment as for instance: temperature variations, CO<sub>2</sub> (pH) changes, micro biota growing on membrane sampler surfaces etc.

The work is an important scientific report and is a novel solution both in terms of a scientific experiment, and the interpretation of the obtained results. In my view, the study should be continued.

I want to indicate that I determine Ganna Fedorova's Doctor of Philosophy thesis outstanding. Therefore, I would like to express my very good opinion about the thesis submitted for the review.



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### **FINAL RECOMMENDATION**

PhD Thesis can be recommended for defence

PhD Thesis can be recommended with reservations for defence

PhD Thesis can not be recommended for defence

4.06.2013

.....  
Date and place

KRAKÓW  
Polsko

Adam Grochowalski

.....  
Name and signature