

REPORT ON THE DOCTORAL THESIS OF

JANA AUFARTOVÁ

The PhD student, Jana Autofartová, from the Department of Analytical Chemistry of the Faculty of Pharmacy in Hradec Králové (Charles University in Prague) has presented a Thesis entitled: “Development of new methods for monitoring of occurrence of pharmaceutical in the environment” in the framework of a collaboration between this University and the University of Las Palmas de Gran Canaria (Spain), under the supervision of Prof. Petr Solich and Prof. José Juan Santana Rodríguez.

The Thesis has been rigorously planned and organized and the objectives are clear and concise. The results are innovative and provided an outstanding scientific contribution in the emerging field in analytical chemistry related with the monitoring of pharmaceuticals in environmental samples, considering the concern about possible negative effects for humans and animals exposed to this contamination. Important groups of pharmaceuticals have been considered: antibiotics (fluoroquinolones), endocrine disruptor compounds (steroid hormones) and fungicides (benzimidazoles), currently used in human or veterinary medicine.

The developed contributions have made possible the publications of some scientific papers in analytical chemistry journals of high impact factor in JRC citation index (3 research articles and 2 review articles) and 1 book chapter, demonstrating the quality of the experimental work carried out. Also 10 poster presentations and 1 poster award have been obtained.

Theoretical part

1. Introduction and Pharmaceuticals in the environment

A brief overview has been properly developed about the problem of pharmaceutical compounds in the environment and specifically about the three families of compounds (fluoroquinolones, steroid hormones and benzimidazoles), their occurrence in the environment, the problems associated, including references.

Some remarks to this part:

- p. 12: Regarding the sentence. “.. some antibiotics are used as growth promoters...”, it should be good to indicate that in the present this is not allowed because this practice was forbidden in since first of January 2006 in consequence of the last Feed Additives Regulation (Regulation (EC) No. 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. Official

Journal of the European Union L 268, 18 October 2003, pp. 29-43). Also this fact must be considered in p. 18, when again candidate indicates their use as growth promoters.

- P. 13: “2.4. Recent development of analytical techniques”

In my opinion I think this title is not appropriate for this part because the candidate is only including briefly the commercial advance in UHPLC and the development of new columns. Also, I think that this part should be named in other way or also to contain advances in other methodologies used for these kinds of compounds as previous works. It is also true that some of these references have been included in the presented reviews and other parts but I consider that the content of this part maybe is not reflecting the title.

2. Sample preparation in environmental analysis

This part describes briefly the most important aspects of some methodologies for sample treatment, including the most recent ones related to microextraction techniques, used for the analysis of environmental samples, as well the advantages of experimental designs in the optimization of relevant variables, including some references.

Some comments to this part:

- p. 34 and Figure 5: I think there is a little mistake in the scheme of DLLME in figure 5. As the candidate is indicating in p. 34, in this technique the mixture of extractant solvent and disperser is rapidly injected into the sample, to create turbulence. However in the scheme, she is indicating that the extractant is included in the vial with the sample and only the disperser is injected.
- Maybe it should be good to include some references or reviews about examples of applications of this methodologies in the monitoring of these kind of contaminants included in this Thesis, just to know the antecedents.

3. Results and discussion

This part is summarizing the experimental work carried out, including also more extended part related to contributions in the field because of some reviews have been produced in this Thesis.

One remark:

- p.49: Table 9 includes published studies for fluoroquinolones by using SPE. I think it should be more appropriate to say that candidate is only selecting methods using LC or UHPLC with MS detection because there are more methods for FQ in water samples using other techniques, such as CE, or other detection methods, such as fluorescence or LIF.

Experimental part

Supplement 1: determination of fluoroquinolone antibiotics using UHPLC with MS and fluorescence detection

The candidate proposes a useful method for UHPLC-MS/MS of fluoroquinolones and also a new methodology to work with SPE using alkaline OH, comparing also the results with UHPLC-FD detection. The method seems to be very sensitive for MS detection and suitable for water samples.

Questions:

- In the validation process, considering the complexity of waters from wastewater treatment plant (WWTP), is it not necessary to calibrate in presence of matrix or with standard solution previously treated with the SPE procedure just to correct possible systematic errors?
- Considering the power of UHPLC-MS/MS: what was the reason to select these 5 FQs? Is it possible to include more quinolones in the analysis to be retained by the proposed SPE treatment? The selected FQs are of human use but you know that you could also find in environmental waters some residues of other used in veterinary medicine (apart from CIPRO and ENRO).

Supplement 2: Determination of steroid hormones in biological and environmental samples using green microextraction techniques

Very useful review containing 75 references and including an important trend in analytical chemistry, the use of more environmentally friendly techniques for sample analysis, classified as green techniques.

Question:

- From all the techniques summarized in this review, what do you think that allows the best characteristics for sample treatment in the case of analysis of these compounds?

Supplement 3: High-sensitivity analysis of female hormones in environmental samples

It includes a critical review with clear and complete tables about the analysis of female hormones containing information about different analytical techniques and different strategies to improve sensitivity, mainly in relation to sample treatment and detection techniques.

Supplement 4: Optimization of an in-tube SPME method coupled with HPLC for the determination of some oestrogens in environmental liquid samples using different capillary column

This paper presents a novel contribution related to the use of a green technique for sample treatment. The results are satisfactory in terms of preconcentration factor and detection limits for water samples.

Some comments:

- You are indicating the flow rate of the sample aspiration by the injector (0.31 mL/min). Can you control this flow or this is the flow imposed by the injector?
- There is a little mistake in Table 2: sample volume is expressed in mL but in fact this is in μL .

- How have you estimated the recoveries of table 4, considering that using the in-line SPME you are preconcentrating?
- How about the reusability of the capillary columns?

Supplement 4: Development of a novel in-tube solid phase microextraction based on micellar desorption followed by LC-DAD-FL for the determination of some endocrine disruptor compounds (EDCs) in environmental liquid samples

Excellent and innovative contribution about in-tube SPME by using another configuration in comparison to the proposed one in the previous paper and micellar medium for desorption.

Question:

- What are the main differences between both procedures for applying the in-tube SPME (in this cases I realize that you are substituting the loop of the injector with the GC column and also you are using manual injection in spite of automatic injector, for example)? Also, what are the advantages and inconvenient of both modes of operations?

Supplement 5: Benzimidazol Fungicides in environmental samples: extraction and determination procedures

Useful book chapter containing excellent tables about the analysis of these compounds, the sample treatment and analytical techniques, kind of sample and references.

After a carefully and deep analysis of the Thesis I consider that this written document has got scientific merit for approval and discussion as a PhD Thesis and the candidate has demonstrated his capability in carrying out analytical work.

Granada, May 15, 2012

Prof. Dr. Ana M. García-Campaña