Examiner's Report for PhD Thesis

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Title: Development of novel approaches to automated sample preparation for pharmaceutical and environmental analysis

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The submitted doctoral thesis on novel approaches to automated sample preparation for pharmaceutical and environmental analysis is presented in a format based on candidate's 6 journal articles, importantly and laudably all published or accepted for publication in Q1 journals. The thesis is well presented and formatted, with most of its 93 pages including 185 references dedicated to an extensive theoretical part with literature overview, additionally there are 8 supplements (6 of them are the candidate's journal articles). It is written in English, the candidate expresses herself clearly, and the language is satisfactory (for some corrections see Minor comments and Errata below).

The thesis is logically structured into the following sections: Two brief sections 1. INTRODUCTION (p. 1) and 2. OBJECTIVES (p. 3) are followed by the largest section 3. THEORETICAL PART (pp. 5-51, plus related references 1-183 on pp. 75-91); the results section 4. RESULTS AND DISCUSSION (pp. 53-72) based on candidate's publications, and overall conclusions from the thesis presented in 5. CONCLUSION (p. 73); this is followed by 6. LIST OF OTHER OUTPUTS OF THE CANDIDATE (pp. 75-78), 7. REFERENCES (pp. 79-92), and 8. SUPPLEMENT (p 93).

The short introductory section 1. INTRODUCTION (p. 1) positions the work in the area of flow techniques (FT), briefly introduces the main techniques FIA, SIA, and LIS (Lab-In-Syringe) and positions the thesis as "mostly focused on the automation of the extraction procedures using the LIS technique in different configurations".

The section 2. OBJECTIVES define the main objective of the work as "to explore the possibilities of non-separation flow techniques in sample preparation in environmental and pharmaceutical analysis and for the study of the dynamic leaching of emerging pollutants." and the aim as "to develop automated methods using mostly Lab-In-Syringe technique in various configurations". This is followed by 6 specific objectives, corresponding to the 6 publications from the work.

The theoretical part 3. THEORETICAL PART presents an overview of the field including literature review with 183 references. It is structured into three main sections (chapters): 3.1. Approaches for automation of analytical processes, 3.2. Sample preparation and possibilities of automation, and 3.3. Bioaccessibility studies. The section justifiably deals in appropriate detail with flow techniques, including their brief history, and their contemporary aspects, specifically automation of FTs, approaches to hyphenation of flow techniques with detectors, solid and liquid phase extraction techniques and their automation including the LIS technique, and finally with bioaccessibility studies, where FTs and aspects of automation can be very useful as well. Clearly, this extensive part, impressive in its breadth, demonstrates the candidate's ability to extract and organise relevant information from the literature, and discuss it from a perspective of the own work's objectives and aims.

The experimental part 4. RESULTS AND DISCUSSION presents the results of 6 work-packages (sub-sections within the section 4.). The candidate presents her results in the form of commented published papers. Within the section 4., after the section 4.1. List of publication included in the thesis, the following sections 4.2. - 4.7. provide comments on the papers 1-6 from the thesis. The comments (each 2-4 pages) introduce the published article within the context of the thesis. Importantly, in this section the candidate shows her ability to present, explain and discuss ideas. This is very significant and speaks of the candidate's capability of communicating her work.

The part 4.2. (Publication 1., Talanta, 2017) describes a proof-of-concept development and demonstration of automation of LIS-DLLME with oxidative back-extraction of metals (Cd, Cu, and Pb) to ICP-AES. The method allowed fully automated sample clean-up and preconcentration of the selected metals from complex matrices including seawater, surrogate gastric juice, and soil leachates.in elemental analysis in difficult matrices, which obviously has a great practical significance.

The part 4.3. (Publication 2., Anal. Chem., 2017) presents work that evolved from the work in the previous part. Here the automated LIS-DLLME was for the first time on-line coupled to ICP-AES with direct injection of the organic phase extract i.e. without posterior back-extraction. In comparison to LIS-DLLME with the back-extraction step (Talanta, 2017), higher enrichment factors thus lower limit of detection were achieved here with a simpler procedure, albeit requiring a specialised ICP instrument.

The part 4.4. (Publication 3., Talanta, 2018) describes the development of an automated method based on DI-SDME by LIS for the determination of nanomolar concentrations of lead using simple and portable instrumentation. Even with reported limits to the analysis speed because of longer extraction time, thanks to the automation of the whole process, sample throughput in the order of 10 samples per hour was still achieved.

The part 4.5. (Publication 4., Talanta, 2019) reports a for the first time developed automated continuous flow in-syringe DLLME with posterior back-extraction and spectrophotometric detection of nitrophenols. An interesting aspect of this work was the multivariate spectral analysis used to distinguish between different isomers of mono-nitrophenol (ortho-, para-, meta-nitrophenol) and thus avoid their separation by chromatographic techniques. This work also collected a Best Poster prize at the 21st ICFIA 2017 (Saint Petersburg).

The part 4.6. (Publication 5., Talanta, 2020) presents a for the first time developed LIS-automated SALLE with on-line SPE and HPLC separation in one closed system for the determination of sulfonamide antibiotics in urine. Achieving synchronisation of different parts of an overall automated system is generally very challenging.

The part 4.7. (Publication 6., J. Chromatogr. A, 2019) presents for the first time realised automatic dynamic leaching of plastic additives from microplastic materials into seawater studied using a flow-based platform hyphenated to on-line liquid chromatography. The work has obviously a potential for use in relevant environmental studies, while making use of the fully automated nature of this method.

The work presented in this thesis is in an area of modern analytical chemistry of high significance in the context of automated flow analysis. The high novelty, impact and overall quality of the outputs is witnessed by 6 journal publications (of those 5 have been already

published and one accepted for publication) in quality (Q1) journals in the area of analytical chemistry (the last 3 publications as the first author), and thus very well satisfying the standards expected for a PhD. Presenting thesis by publications means the findings were already assessed by professionals in the field to the highest standards including judging novelty and impact of the works. Hence the corresponding work in this thesis can be accepted as scientifically sound and of satisfactory quality.

Overall, the experimental work presents significant new concepts and ides. Importantly, the candidate shows an ability to present, explain and discuss ideas, which is very important, and speaks of the candidate's maturity as a scientist capable of communicating her own work.

The methods are technically challenging, but well executed as in this work, they appear surprisingly elegant, even simple, and are relatively low-cost (except specialised analytical instrumentation such as ICP), rapid, and flexible. It needs to be pointed out that the experimental work in the area of modern instrumental analytical chemistry is very technical and thus demanding high experimental skills, therefore its successful completion witnessed by the 6 journal publications documents the candidate's proficient experimental capabilities.

The investigated a wide range of analytes from inorganic ions (heavy metals) to small organic molecules with significance as environmental pollutants and pharmaceuticals is of considerable practical significance and adds to the already significant span of this work.

In conclusion, in my view the submitted thesis is of an overall outstanding quality, fulfils the requirements, and can be recommended for the defence, and following a successful defence, the degree PhD may be awarded.

Finally, if I had an opportunity to ask the candidate a question about her PhD work, I would ask one of the following questions:

1. What are the principal and technical differences between sequential injection chromatography (SIC) and liquid chromatography (LC, HPLC)?
2. Have you (with your collaborators) considered reworking the theoretical part of your thesis into review papers?
3. Have you (with your collaborators) considered protecting intellectual property in elements of your work, and commercialisation of those parts?

Minor comments and Errata:

1) In the sections 4.2. - 4.7., under the publication title, the literature reference should be given. This would save the reader from having to keep going back to the list of publication in 4.1. on pp. 53-54 to see which journal was each paper published in and when.
2) "CONCLUSION" should be "CONCLUSIONS" (unless only one (1) conclusion is presented). Similarly SUPPLEMENTS, List of publications, Comments on publication 1, etc.
3) Terminology: "dead volume" (p.67, and elsewhere in the thesis) would be better "void volume".