

## **PhD Dissertation: Application of non-separation flow techniques in pharmaceutical analysis**

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### **External report:**

The PhD dissertation reports recent developments in the field of automation of sample processing methods exploiting advanced flow methodology and batch-flow type systems, including in-syringe setups, for determination of parameters of pharmaceutical and biomedical interest with further extension to environmental applications.

The PhD dissertation is breakdown in four main sections. The objectives are clearly defined and signaled the ambitious research proposal of this PhD thesis. The introduction is very well written and easy to follow. It is divided in three subsections. Selected sample handling procedures encompassing several liquid-phase based microextraction procedures and sorbent-based approaches (including microextraction procedures) are described in the first subsection. I missed however some quotes of well established solid-phase microextraction procedures (e.g., needle-trap methodologies) or solidification of floating organic droplet as a liquid-phase microextraction alternate. The author should avoid some categorical statements when referring to extraction techniques. For example, there is a plethora of recent papers reporting new materials for stir-bar sorptive extraction to expand the applicability scope from the classical PDMS sorptive phase (p. 19). Another example is found in the very same page: SPME is not restricted to analytes with high vapor pressure as this approach is not merely restricted to GC applications.

Illustrated with appropriate close-ups and schemes the second sub-section nicely summarizes the fundamental principles and merits of the various generations of flow injection including the main components thereof. To set the research undertaken into context, the third introductory sub-section details previous research works capitalizing on the use of enzymatic reactions, external energy sources and solid-liquid and liquid-liquid microextraction approaches in flow systems in a variety of research arenas. Though not fully comprehensive, more than 250 references have been quoted to showcase the current state-of-the art in employing flow-based approaches in pretreatment of samples with distinct degrees of complexity. At this juncture, I wish to stress that the literature is updated as demonstrated by the compilation of about 60 references published over the past three years. The various Tables in the introduction listing the analytical performance of literature papers are deemed most useful to readers for grasping previous research efforts and endeavors in the field.

The core of the PhD dissertation is composed of six -already published- scientific papers subjected to rigorous peer-reviewing with five of them published in first-tier analytical periodicals according to the Journal Citation Reports (JCR). I liked the idea to include a brief introductory comment to each publication so as to indicate the novelty of the research work and summarize the most salient findings. The versatility and quality of the PhD dissertation is clearly reflected in the variety of sample handling processes automated via flow-based approaches ranging from enzymatic reactions in the liquid phase, micro-solid phase extraction

(MEPS format) coupled to moderate pressure chromatography (SIC) to batch-flow configurations accommodating liquid-phase microextraction, single-drop head-space microextraction and fluorescence detection of pesticide photoderivates following preconcentration and UV-decomposition. Further, five out of the six publications are the result of scientific collaboration and short research stays of the candidate at the University of Porto and University of the South (Bahia Blanca, Argentina), thereby demonstrating the internationalization of the activities undertaken.

The last section is supposed to summarize the conclusions of the dissertation. As far as I am concerned this is the weakest point of the overall thesis as it merely contains half a page of general comments about flow approaches without any quantitative data or experimental finding. A critical view of the pros and cons of developed methods is missing along with the author's own perspective as to where the field of automatic sample processing using flow methods is directed.

There are three more issues that the PhD candidate might wish to address:

1-I missed some critical comparison of the distinct on-line/in-line microextraction approaches used in the dissertation: LPME, MEPS, sorptive SPE column and single drop liquid microextraction in terms of analyte absolute recoveries, matrix effects, extraction times, enrichment factors, intermediate precision, calibration procedures, etc...

2-Insights into the mechanism of UV-irradiation and nature of the fluorescent photodegradation product from metsulfuron methyl might be added to support experimental findings.

3- The reasoning for evaluation of the experimental variables in the overall papers by univariate methods rather than experimental designs followed by surface response methodologies should be indicated. Univariate methods might provide some rough estimation of the influence of variables on the analytical response but cannot be generally used for optimization procedures, e.g., finding maximum or minimum values.

On the basis of the scientific quality and novelty of the research compiled in the PhD dissertation I am pleased to recommend Mrs Sramkova to be awarded with the PhD degree.

July 26, 2015

A handwritten signature in yellow ink, appearing to read 'Manuel Miró', is written over a faint, light-colored rectangular stamp or watermark.

Manuel Miró