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EXTRACTION STRATEGY FOR TARGET AND NON-TARGET ANALYSIS OF ENVIRONMENTAL CONTAMINANTS IN BIOLOGICAL MATRICES USING GC-MS/MS AND LC QTOF-MS/MS

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Introduction

Our developed, highly technological society has more than 100,000 chemical substances registered with several hundred new chemicals being introduced and registered every year [1]. Because of the potentially adverse environmental and/or health outcomes associated with exposure to such chemicals, data concerning the presence and the concentration of these chemicals in biological matrices is needed. For several decades, target analysis have been mainly use to analyse contaminants in biological matrices [2]. The drawbacks of this technique is that only targetted chemicals can be detected and quantified meaning that a lot of other chemicals potentially toxic or present in high concentration will be ignored. Analytical methods for a rapid and sensitive screening of a broader range of compounds in complex biological matrices are required.

Stepping towards a more comprehensive assessment of human and biota exposure to contaminants, we present in this work the development and optimization of an analytical strategy that combines efficient and reliable extraction, purification and analysis of a broad range of polar and non-polar target analytes in fatty biological matrices including breast milk and fish muscle. Our method combines targeted multiresidue analysis using gas chromatography triple quadrupole mass spectrometry (GC-QqQ-MS/MS) and a multi-targeted analysis complemented with non-target screening using liquid chromatography coupled to a quadrupole time of flight mass spectrometry (LC-QTOF-MS/MS).

Using the optimized method, 9 samples were extracted and analysed. After blank substraction several targets, suspects and non-target compounds could be identified.

Materials and methods

To extract a wide range of chemicals, the partition/extraction procedure used for the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) was used as the initial step for the extraction. The method development was done by using a broad range of target analytes from various chemical groups. All the target model analytes have different physico-chemical properties and cover a broad activity spectrum (log Kow ranges from -0.3 to 10); from polar pesticides, pharmaceuticals, personal care products (PPCPs) to highly lipophilic chemicals such as polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and organochloride pesticides (OCPs).

For sample preparation the QuEChERS (quick, easy, cheap, effective, rugged and safe) method was tested, samples were extracted using acetonitrile and salts for phase separation, followed by a sample clean-up using dispersive solid phase extraction and protein-lipid removal filter cartridges (Captiva ND Lipids).

Our proposed strategy is based on the combination of a GC-MS/MS and LC-QTOF-MS/MS analysis.

Results and discussion

-Method optimisation

A number of options were explored for the clean-up of lipids, proteins and other impurities present in the matrix. Parameters tested were the control of the pH during the extraction process (by addition of formic acid at 0.1, 1 and 2% as well as the use of buffer) and the clean-up efficiency provided by different dispersive solid phase extraction sorbents (ZSep, PSA + C18, ZSep/C18) and by the protein-lipid removal filter cartridges Captiva ND Lipids. Zirconium dioxide-based sorbents as dispersive solid-phase extraction (d-SPE) and protein-lipid removal filter cartridges (Captiva ND Lipids) provided the best results for GC-MS and LC-MS analysis, respectively. The optimized conditions are summarized in the figure 1.

-Method validation
The method was fully validated for samples of fish muscle and breast milk through the evaluation of recoveries, matrix effects (ME), limits of quantification (LOQs), linearity and precision (interday and intraday). Recovery experiments were conducted at a spike level of 8 µg/L (for musks, benzophenone, PAHs, PCB and PBDE congeners) and 20 µg/L (all other analytes). Mean recoveries (n=5) were between 70% and 120% with relative standard deviations less than 20% in most of the cases. The good recoveries obtained for the chemicals with a wide range of log KOW show the applicability of the method to extract a broad range of chemicals (Fig. 2). GC-MS/MS LOQs ranged from 0.08 to 3 µg/kg and LC-QTOF-MS/MS LOQs ranged from 0.2 to 9 µg/kg. In GC-MS/MS, ME were lower than 20% for 80% of the chemicals in fish extract and for 65% of the chemicals in breast milk. Matrix effects observed in LC-QTOF-MS/MS analysis are relatively similar in fish and breast milk extracts. More than 61% of chemicals presented ME lower than 20%. In general MEs were highly variable and up to 35% of the chemicals presented a matrix effect > 20% demonstrating the importance of using matrix-matched calibration to improve the accuracy of the quantification for LC-QTOF-MS/MS and GC-MS/MS analysis.

-Method application to real samples
To demonstrate the applicability and suitability of the validated method, 4 fish samples and 5 breast milk samples were analysed.
The results showed that of the 77 target compounds monitored, a total of 29 were quantified in the analysed samples. The present work also demonstrated the feasibility of discovering untargeted compounds in real samples using the developed extraction method in combination with LC-QTOF-MS/MS analysis. Besides the identification and quantification of several target compounds in the real samples, other new contaminants have been identified in the fish and breast milk samples, such as the transformation products of widely used insecticides and fungicides, a flame retardant, PFOS and preservatives and its metabolites.

Conclusions
In this study, a modified QuEChERS procedure followed by analysis with GC-MS/MS and LC-QTOF-MS/MS has been demonstrated to be a powerful methodology for the simultaneous analysis of a wide range of chemicals with different physico-chemical properties in breast milk and fish extracts [3].
The developed method is simply and fast, and good results were obtained in terms of sensitivity, recoveries and precision. In addition to multi-target analysis of fatty biological matrices, this strategy has shown to be suitable for non-target screening, given a more comprehensive view of the true overall contaminants present in the samples.
The developed method can potentially be applied for other biological matrices; we have already used this method successfully in the analysis of bird tissue and with some modifications in blood from human and biota.

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References
[1] Geiss F et al. (1992), Toxicological & Environmental Chemistry. 37, 21-33.
[2] Rodil J. B et al. (2009). Journal of Chromatography A, 1216, 2958-2969.
[3] Baduel C et al. (2015). Journal of Chromatography A, 1426, 33-47.

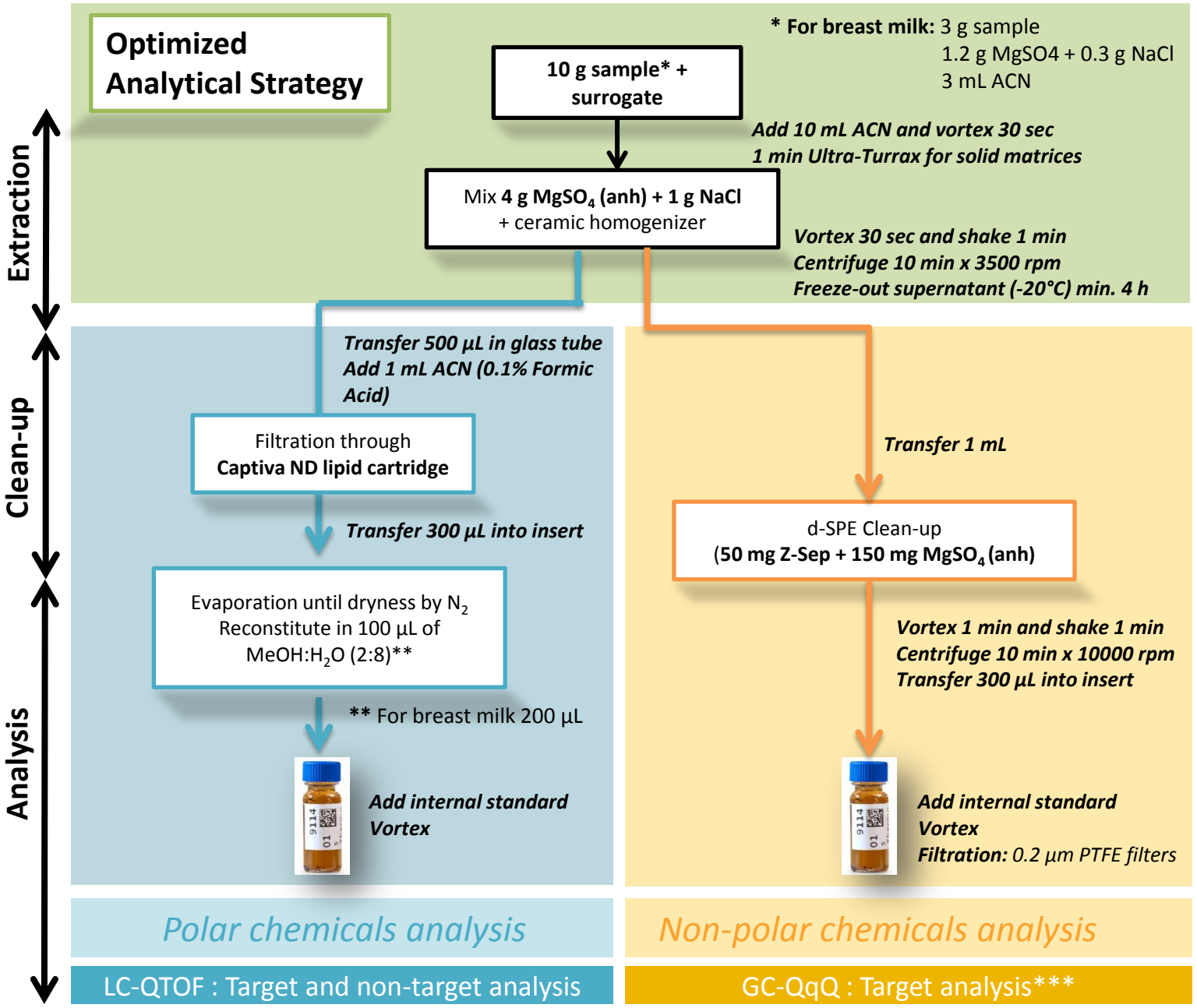

Figure 1. Optimized analytical approach for the extraction and analysis of polar and non-polar compounds in biological matrices with low fat content

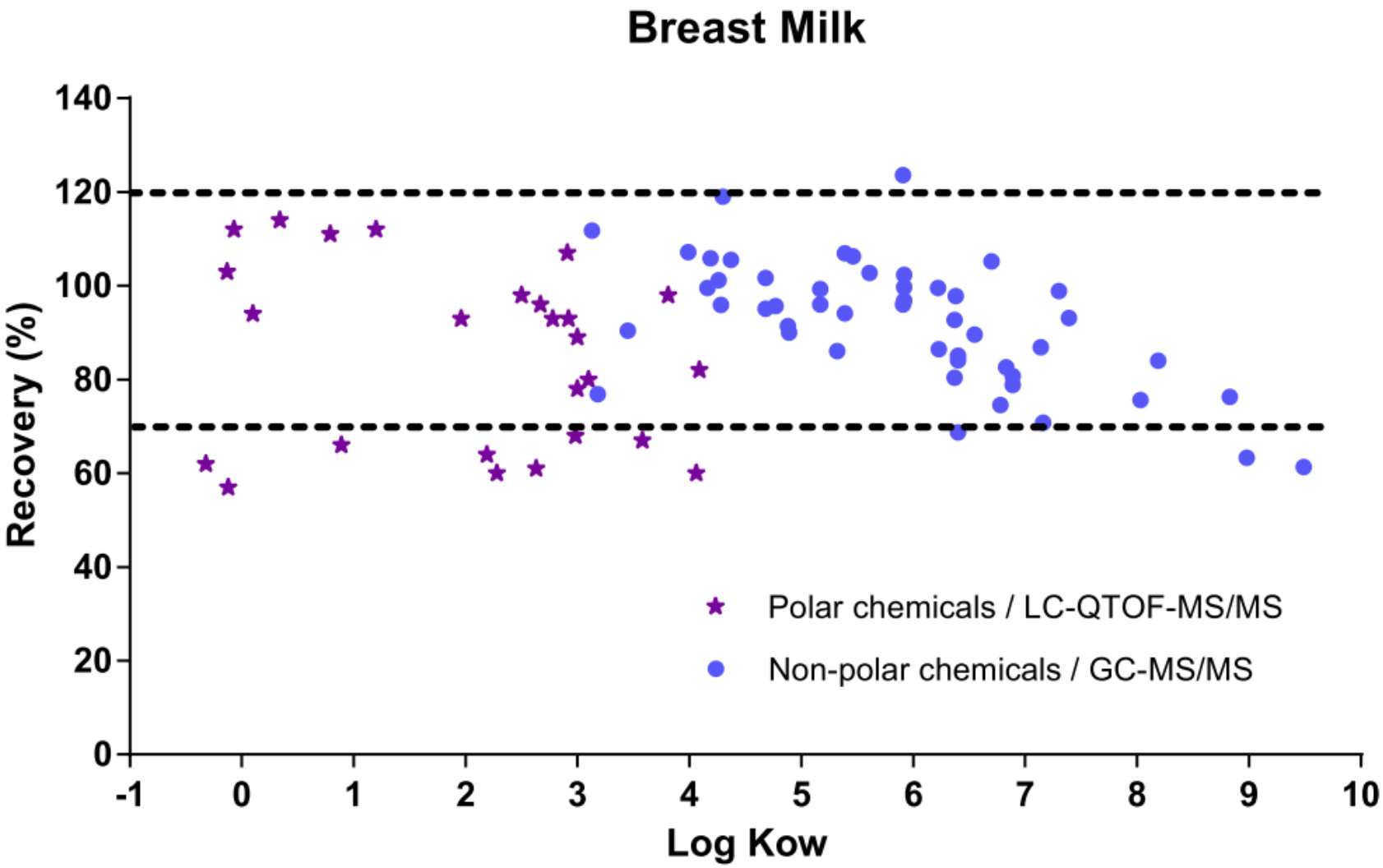

Figure 2. Recoveries of targeted compounds related to their Log KOW values using the optimized procedure for breast milk samples