# DEVELOPMENT AND VALIDATION OF A MULTI-METHOD FOR THE ANALYSIS OF 33 MYCOTOXINS AND 149 PESTICIDES IN COCOA BEANS USING UPLC-MS/MS ($ESI^+$)

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Due to the problems related to serious fungal diseases faced nowadays by cocoa producers, an increase on the level of mycotoxins found in this cocoa beans and its processed products is expected. Associated with that, higher levels of pesticides, especially fungicides, are also likely to be found. Considering this situation, this work has the purpose to compensate the lack of knowledge on mycotoxins and pesticides analysis with the development and validation of a method able to identify and quantify those substances in monitoring analysis.

During this study, various clean-up procedures were tested with a modified QuEChERS method [1] which was optimized to analyze mycotoxins and pesticides simultaneously. Due to unacceptable losses, a clean-up step was omitted for the final method. Deletion of the clean-up step was proven to have insignificant influence on the matrix effect and robustness of the analysis [2].

Cocoa beans were homogenized via an efficient slurry procedure (sample/water ratio 1:3 w/w) and then 10 g slurry were extracted with acetonitrile/acetic acid 1%, followed by a partitioning step, induced by magnesium sulphate addition. The pH stabilization was done trough the sodium acetate addition in combination with the acetic acid already present. An aliquot of extract was removed before the buffering step for mycotoxins analysis to avoid losses.

For validation purposes, the mycotoxins were divided in two groups. One group with the 17 mycotoxins which were detectable more sensitively at the spiked level of 1, 2 and 10 µg $kg^{-1}$ and other group with the 19 less sensitive ones spiked at 50, 100 and 400 µg $kg^{-1}$. All pesticides were spiked at 10, 20 and 50 µg $kg^{-1}$. All compounds were analyzed six times under repeatability conditions for each spike level. Linearity of analytical curves, recoveries, precision (RSD%) and matrix effects were determined.

From the 33 mycotoxins analyzed, 21 presented recoveries between 70% and 120% and RSD lower than 20% and from the 149 pesticides, 116 had results in the same range and were successfully validated. Five of the mycotoxins with a LOQ reaching 1 and 2 µg $kg^{-1}$, the other with values between 5 and 100 µg $kg^{-1}$. For pesticides, 57% of them with a LOQ of 10 µg $kg^{-1}$, 14% of 20 µg $kg^{-1}$ and 7% of 50 µg $kg^{-1}$, the others couldn't be quantified reliably. Matrix effects were significant, but had no considerable influence on the obtained results.

The method developed has been applied successfully for a survey of mycotoxins in cocoa beans. The compounds detected prove the relevance of the method.

[1] Pizzutti, I. R., De Kroon, M., Prestes, O. D., Rensen, P., De Kok, A., 7th European Pesticide Residue Workshop, Berlin, Book of Abstracts, 2008, 213.