

USE OF IMMUNO-AFFINITY COLUMNS CLEANUP FOR CHLORAMPHENICOL SCREENING ASSAY IN FOOD SAMPLES

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Since 2003, our laboratory has carried out the analysis of samples of milk and dairy products for the presence of residual amounts of chloramphenicol. In the initial stages of the research, the samples of various types of condensed milk with sugar and hard cheeses have been tested by LC-MS/MS as more sensitive and specific. However, due to a significant increase of the amount of routine work, the question has appeared about the optimization of the sample preparation sample and the possibility to involve the immunoassay to the research.

This method is almost as sensitive as LC-MS/MS technique and allows simultaneous analysis of a large number of specimens in a short time without the need for multistage purification during sample preparation. The specificity and selectivity of ELISA directly depends on the ability of the specific antibodies to bind the analyte and on the possible non-specific binding of the antibodies to other components of the sample matrix.

Therefore, the sample preparation for LC-MS/MS analysis was used as a basis for ELISA, viz. the extraction of the analyte with ethyl acetate and the following concentration by means of drying on a rotary evaporator. After the reconstitution of dried residue, Easy-Extract Chloramphenicol immune-affinity columns (R-Biopharm) were used to purify and concentrate the extracts.

The percentage of extraction for samples of various types of condensed milk with sugar was in the range of 47 to 111%; for butter samples – from 54 to 74%, and for hard cheese – from 37 to 47%. It suggests that the purification and concentration method using immune-affinity columns can significantly reduce the matrix effect on the ELISA system, despite the sample composition, thus minimizing non-specific adsorption of the matrix components for immunity studies of the analyte.

The intra-laboratory validation was carried out to establish the effectiveness of an immune-affinity column clean up procedure followed by ELISA for the determination of chloramphenicol in cheese, condensed milk with sugar, condensed

milk with sugar and cocoa, condensed milk caramel with sugar, and butter. The results were compared with the criteria, according to Decision 2002/657/EC.

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