CLARIFYING INSTRUCTIONS ON THE ANALYTICAL METHOD

SUPPLIED MATERIAL

For the analytical method validation the following material will be supplied, as per the LETTER "SAMPLE PREPARATION":

- Three solutions of Bisphenol A (CAS 80-05-7), each with a different known concentration, to verify the performance parameters of the solvent matrix method.
- Different portions of naturally-contaminated fish paste

HOW TO IMPLEMENT THE ANALYTICAL METHOD

INTRODUCTION

The procedure aims at determining the concentration of Bisphenol A (CAS 80-05-7) in complex food matrices (canned fish and canned vegetables) through the QuEChERS extraction method and the subsequent separation and analysis with LC/MSMS. The quantification is carried out on the basis of an internal standard.

LoQ: 0,01 mg/kg

Measurement range: 0,01 mg/kg - 0,1 mg/kg. Maximum value: 10 mg/kg (considering subsequent sample dilutions)

QuEChERS EXTRACTION (suited for fat rich foods)

UNKNOWN SAMPLE

Weigh 10 g ± 0,1 g of food in a 50 ml centrifuge tube; in case of very concentrated samples, lower the weight to 1g ± 0,01 g. Add 10 ml of water to improve sample dispersion. Add 10 ml of acetonitrile and proceed with all of the QuEChERS method extraction phases. The extract will be analysed using LC MS MS.

CALIBRATION

It is possible to use two different methods:

i. STANDARD ADDITION: use of the contaminated sample for all of the seven calibration points.
Weigh six portions of 10 g ± 0,1 g of food with unknown concentration in a 50 ml centrifuge tube; in case you had to lower the sample's weight to 1g ± 0,01 g, weigh an equal portion also for the calibration. Add 10 ml of water to improve sample dispersion. Add 10 ml of acetonitrile with different spikes to obtain the desired calibration points (e.g. mg/kg 0,001 - 0,005 - 0,010 - 0,025 - 0,060 - 0,080).
Proceed with all of the QuEChERS method extraction phases. The extract will be analysed in sequence after the unknown sample using LC MS MS.

ii. MATRIX-MATCHED: perform the calibration in the matrix using the provided sample "blank".
Weigh six portions of 10 g ± 0,1 g of "blank" food with unknown concentration in a 50 ml centrifuge tube; in case you had to lower the sample's weight to 1g ± 0,01 g, weigh an equal portion also for the calibration. Add 10 ml of water to improve sample dispersion. Add 10 ml of acetonitrile with different spikes to obtain the desired calibration points (e.g. mg/kg 0,001 - 0,005 - 0,010 - 0,025 - 0,060 - 0,080).

Proceed with all of the QuEChERS method extraction phases. The extract will be analysed in sequence after the unknown sample using LC MS MS.

INSTRUMENTAL ANALYSIS

Acquisition method:

- LC-MSMS analysis in MRM mode.

Column and mobile phases:

- Column C18 50mmx2,1mm - 1,8UM or the like.
- Mobile phase A: Water - Mobile phase B: Methanol + 2mM of ammonium acetate.