

**SUPRAMOLECULAR SOLVENT-BASED MICROEXTRACTION AND
CHIRAL LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY
FOR THE DETERMINATION OF HEXABROMOCYCLODODECANE
STEREISOIMERS IN FISH.****A.B. Lara, C. Caballo, M.D. Sicilia, S. Bravo**

*Departamento de Química Analítica. Facultad de Ciencias. Universidad de Córdoba Edificio Anexo Marie Curie. Campos de Rabanales, 14071- Córdoba. España.
qa1rubrs@uco.es*

Hexabromocyclododecane (HBCD) is a brominated flame retardant used as an additive in polystyrene foams for thermal insulation of buildings, in upholstery textiles and in electrical equipment housings. The industrial additive mainly consists of racemic mixtures of three diastereoisomeric pairs of enantiomers, the γ -isomer being the most abundant, followed by the α - and β -ones. In the last years, the abundance of HBCD in the environment has greatly raised, which together its persistence, bioaccumulative properties and toxicity, has made it an emerging contaminant of very high concern. It is often present in fishes, α -HBCD being the most abundant isomer. The composition of HBCD in fish differs from that of commercial products because the HBCD stereoisomers are absorbed and metabolized at different rates and suffer bioisomerization, with a preferential formation of the α -isomer. Deviation of the enantiomeric ratios [ERs, defined as the molar ratio of (+)- to (-)-enantiomers] from their original value (i.e. ER=1 for racemic mixtures) has been also observed for the three diastereoisomeric pairs.

This work deals with the development of a simple and rapid method for the quantitation of the six stereoisomers of HBCD [(+)- α -, (-)- α -, (+)- β -, (-)- β - and (+)- γ -HBCD] in fish. Reported methods for the stereoselective determination of HBCD in fish samples invariably involve liquid chromatography (LC) with mass spectrometry (MS) detection after a multiple-step sample treatment including extraction by Soxhlet or accelerated solvent extraction (ASE) and clean-up by gel permeation chromatography followed by SPE and/or sulphuric acid treatment. The method developed by our research group permitted to determine individual HBCD stereoisomer at concentrations at the low ng g⁻¹ level in fish using a single-step sample treatment approach based on supramolecular solvent-based microextraction (SUSME). The target analytes in fish samples (750 mg) were extracted in a low volume (600 μ L) of a supramolecular solvent (SUPRAS) made up of reverse aggregates of decanoic acid (DeA) and the extracts were directly analysed by LC-MS after dilution 1:1 with methanol. The HBCD stereoisomers were separated on a stationary phase of β -cyclodextrin and quantified in a mass spectrometer equipped with an electrospray ionization source and a triple quadrupole mass analyzer. The SUSME approach permitted to extract ten samples simultaneously in about 20 minutes and it afforded recoveries near to 100% for all the fish species tested (hake, cod, sole, panga, whiting and sea bass). The high extraction efficiency obtained could be explained on the basis of the different interaction mechanisms provided by the supramolecular solvent (dispersion interactions between the hydrophobic moieties of the target analytes and the hydrocarbon chains of the DeA molecules forming aggregates in the SUPRAS and dipole-dipole interactions between the bromine groups of the target analytes and the carboxylic groups of DeA) and the high number of solubilisation sites of the solvent (the concentration of DeA in the SUPRAS is about 0.8 mg μ L⁻¹). Besides efficient, the method developed is accurate, precise, robust and it surpassed to previously reported approaches in simplicity and rapidity.