2.
Objectives

In order to better understanding of the work, it was separated in 2 parts.

2.1.
PART I: Peptide derivatization and complexation with metals

2.1.2.
General Objective

The aim of this part was to develop a new simple, sensitive, accurate and precise method for identifying and quantifying peptides based on simultaneous coupling of elemental and molecular mass spectrometry to multidimensional chromatographic separations.

2.1.2.1.
Specific objectives

- Development and optimization of suited strategies for the labeling of peptides with metal-chelators DOTA-(Lu, Tm and Ho).
- Elucidation, investigation, and optimization of analytical parameters for the (i) accurate and precise quantification of labelled peptides by ICP-MS and (ii) the molecular characterization of these peptides/proteins by MALDI MS.
- Separation of the labelled peptides and analyses by nano-HPLC with UV detection and MALDI MS.
- Comparison of the strategies developed with others established methods for quantification.

2.2.

PART II: Optimization of metalloprotein extraction procedures from environmental samples

2.2.1.

General objective

The aim of this part of the study was to evaluate and standardize the currently applied thermal-extraction method for the purification and quantification of fish bile MT using different reagents and multivariate statistical analyses in a proteomic/metalloproteomic context, since this protein has previously been reported as a potential biomarker in fish bile.

2.2.1.1.

Specific objectives

- Improvement of MT extraction procedure in fish bile and liver samples.
- Evaluation of the use of fish bile in the detection of metal contamination by comparisons with the same analysis in liver, in order to demonstrate fish bile potential as a biomarker by different analytical techniques.
- Qualitative and quantitative proteomic/metalloproteomic evaluation of bile and liver samples using different analytical techniques: SDS-PAGE, UV-Vis spectrophotometry, SEC-HPLC-ICP-MS, MALDI MS and ESI MS.
- Evaluation of biliary proteins in fish bile as potential biomarkers of environmental contamination using MT-induction responses in laboratory-exposed fish, using the statistical tools.
- Evaluation of clean-up effects in both fish bile and liver by vibrational spectroscopy analyses.