OPTIMISATION OF A MICROWAVE ASSISTED EXTRACTION PROCEDURE COMBINED WITH LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY FOR THE DETERMINATION OF TAMOXIFEN AND CYCLOPHOSPHAMIDE IN FISH TISSUES

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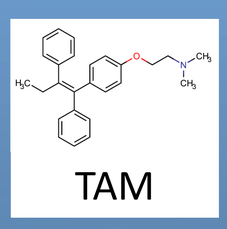
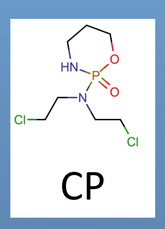
INTRODUCTION

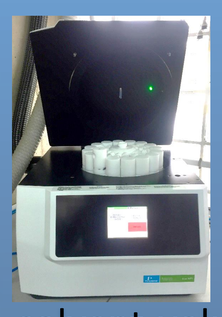
Human activities produce pollutants that come from wastewater treatment plants due to an ineffective treatment in the elimination of certain compounds. Antineoplastic compounds are some of them, which are drugs used as treatments against cancer and can cause important adverse effects if they reach the environmental waters [1]. They have been detected in wastewater and also river water, however, to date, no procedure has been optimized for the extraction of antineoplastic compounds from fish tissue [2].

EXPERIMENTAL

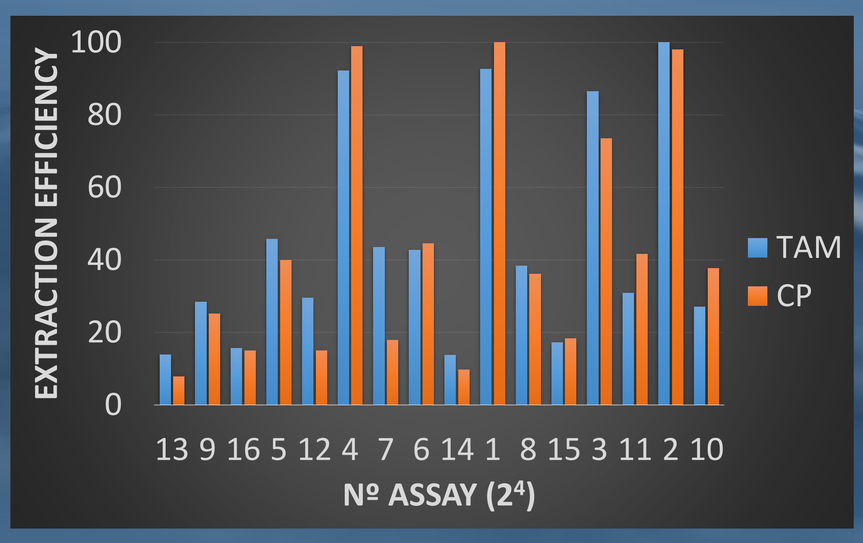
A microwave assisted extraction (MAE) followed my Ultra-High Performance Liquid Chromatography tandem Mass Spectrometry (UHPLC-MS/MS) procedure was optimised with muscle from *Boops boops* .

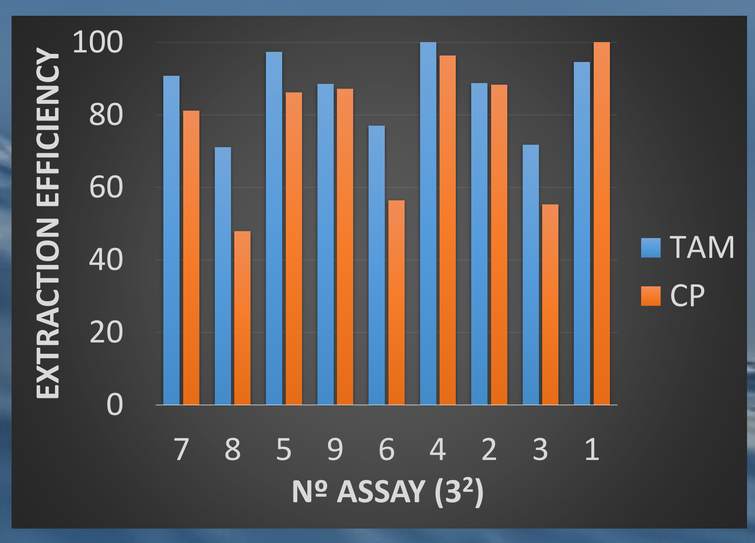






First, an experimental design 2^4 was performed with 4 variables (temperature, time, solvent volume and sample weight) at 2 levels. Then a 3^2 experimental design was carried out with the variables temperature and sample weight at three levels. Finally, the optimal conditions were: 50 mg of fish; T=55ºC; time=5min; solvent volume=7mL of MeOH, clean-up step, dried and reconstituted in 1mL. Analytical parameters were performed at 4 concentrations levels: 0.2, 0.5, 2 and 5 $\mu g \cdot g^{-1}$.





	TAM	CP
Recovery (%)	74 – 95	106 – 122
Matrix effect (%)	-86 – 48	-34 – 46
RSD Intraday (%)	<14.4	<19.4
RSD Interday (%)	<16.8	<20.4
LOD ($ng \cdot g^{-1}$)	0.8	1.3
LOQ ($ng \cdot g^{-1}$)	2.7	4.8

APPLICATION

Different fish were analysed corresponding to different levels of the trophic chain caught in the vicinity of three submarine outfalls of the island of Gran Canaria. No antineoplastic compounds were detected. However, to prove the applicability of the method, muscle and liver of each of them were spiked at a concentration of 0.5 $\mu g \cdot g^{-1}$ obtaining the recoveries showed in the table:

Fish recovery (%)	Tissue	TAM	CP
 *Boops boops*	Muscle	95	122
	Liver	111	91
 *Sphoeroides marmoratus*	Muscle	105	124
	Liver	111	93
 *Sphyraena viridensis*	Muscle	75	109
	Liver	79	93

CONCLUSIONS

- **A method for the determination of antineoplastic compounds from fish tissue has been developed, validated and applied for the first time.**
- **To our knowledge, this is the first time that MAE is applied for the extraction of antineoplastic compounds from solid matrix.**
- **In optimal conditions, we are able to extract 12 samples in 5 minutes, with good recoveries for different fishes and achieving ng levels of some of the most widely used antineoplastic drugs.**

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