16th Annual Workshop on Emerging High-Resolution Mass Spectrometry (HRMS) and LC-MS/MS Applications in Environmental Analysis and Food Safety
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First determination of Carbamazepine emerging pollutant and your metabolites in *Anemonia sulcata* and *Actinia equina* species by ultra-high-performance liquid chromatography Mass Spectrometer (UHPLC-HRMS) and a quadrupole-time-of-flight (QqTOF)

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INTRODUCTION

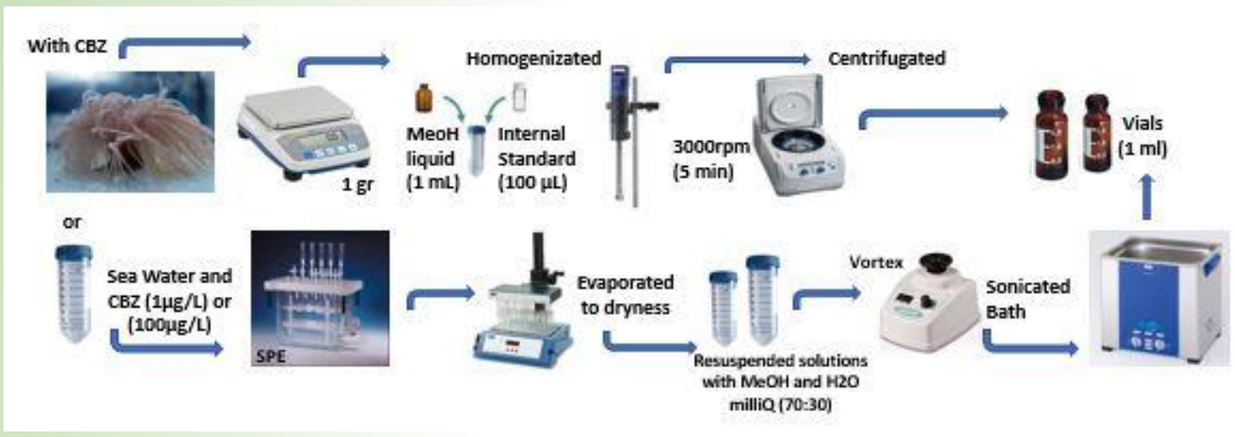
Carbamazepine exposed individuals of anemone such as, *Anemonia sulcata* and *Actinia equina* species were analyzed for the identification of the potential metabolites formed. *A. sulcata* and *A. equina* species are widely distributed in the Mediterranean Sea. Their high tolerance to stress makes they "ideal species" for the monitoring of environmental contamination.

Anemonia sulcata

Actinia equina

MATERIAL AND METHODS

Figure 1: Methodology applied for chemical metabolite determination

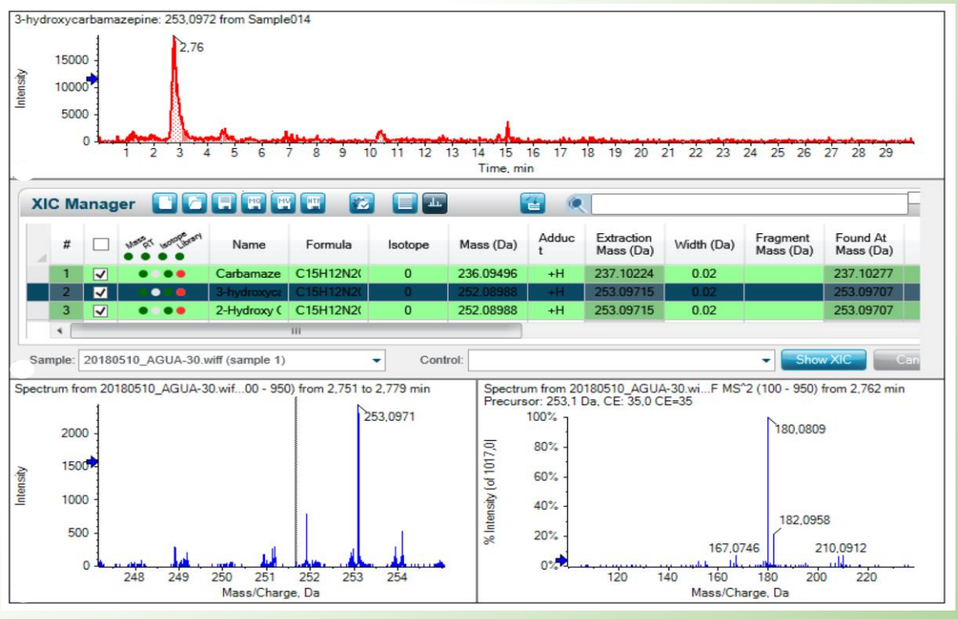


The method to determine CBZ in water was previously published as multiresidue methods but some validation was performed because in this case only CBZ was determined. The extraction of CBZ of animal tissues did not required further clean up or filtrate phase.

LC-MS/MS Analysis

A 1260 Infinity Ultra-High-Performance Liquid Chromatograph (UHPLC), coupled to an Agilent 6410 Triple Quadrupole (QqQ) Mass Spectrometer (MS/MS) (both from Agilent Technologies, Santa Clara, CA, USA).

Figure 2: One example of 3-HydroxyCBZ metabolite plausible molecular formula obtained with HRMS and MS/MS data acquisition.



RESULTS AND DISCUSSION

Table 1. CBZ and CBZ metabolites identified or not by HRMS and HRMS/MS data. The metabolites that appear in bold were detected in *A. sulcata* samples. The others correspond to metabolites detected in water samples.

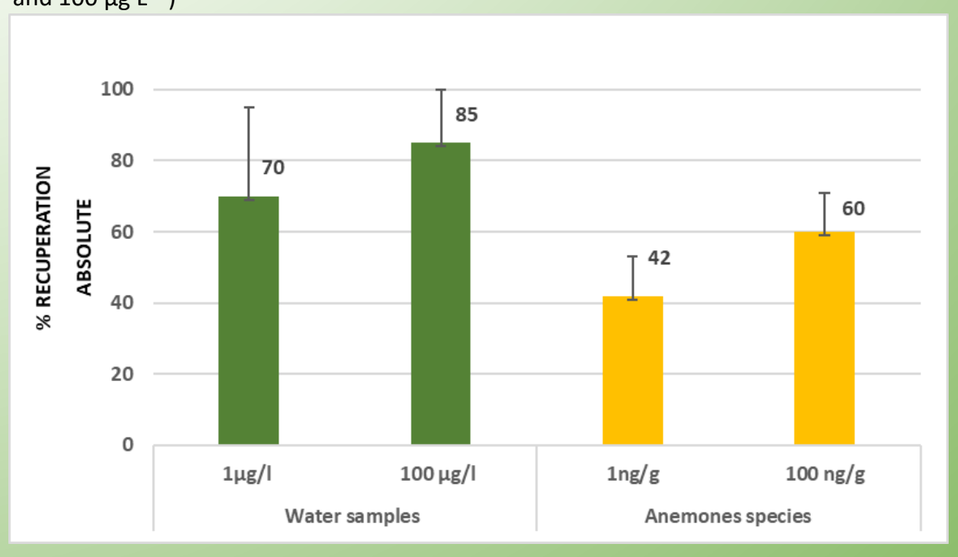
Compounds	Retention time	Formula	m/z teoric	m/z experimental	Error ppm	MS/MS (mz) experimental
Carbamazepine 2,6-pyridinedicarboxylic acid	**10.52**	**C22H17N3O5**	**404,1241**	**404,12437**	**0,7**	**344,1018**
Carbamazepine Nicotinamide	**15.9**	**C22H17N3O5**	**359,1502**	**359,15119**	**3**	**294,0965**
Carbamazepine	**6.92**	**C15H12N2O**	**237,10224**	**237,10279**	**2,3**	**194,0963**
10-Methoxycarbamazepine	0.8	C16H14N2O2	267,1128	267,11301	0.6	198,1042
Carbamazepine-o-quinone	5.93	C15H10N2O3	267,07642	267,07637	-0,2	235,2011
11-Keto oxcarbazepine	6.05	C15H10N2O3	267,07642	267,07601	-1,5	229,2025
3-hydroxycarbamazepine	**2.76**	**C15H12N2O2**	**253,09715**	**253,09707**	**-0,3**	**180,0809**
2-hydroxycarbamazepine	5.18	C15H12N2O2	253,09715	253,09715	0	180,1425
Carbamazepine 10,11-Epoxide	**5.18**	**C15H12N2O2**	**253,09715**	**253,09737**	**0,8**	**229,1918**
Carbamazepine Butyric Acid	10.51	C19H20N2O3	325,15467	325,15425	0	320,0904

Table 2: The bioconcentration factor (BCF in L kg−1) in *A. sulcata* and *A. equina* exposed to CBZ. Measured values given as means ± SD.

	CBZ (µg L^{-1}) (nominal)	CBZ (µg L^{-1}) (measured)	CBZ (µg kg^{-1}) (in anemone)	BCF CBZ (L kg^{-1})
A. sulcata	1	0.21 ± 0.03	0.135 ± 0.03	0.65
	100	74 ± 7.8	1170 ± 179	29
A. equina	1	0.22 ± 0.04	0.113 ± 0.09	0.62
	100	74 ± 8.9	1059 ± 64	24

Absolute recovery ranged from 70% to 85% with RSDs b20% and matrix effects b20%. Method validation pointed out that the LODs were 3 ngmL^{-1} for water and 50 ng g^{-1} for *A. sulcata* and *A. equina* and the LOQs were 10 ng mL^{-1} water and 150 ng g^{-1} for biota. Despite the low amount of biomass considered (10–50 mg).

Figure 3: Recoveries (%) of the target compounds at 2 concentration levels (1 µg L-$^{-1}$ and 100 µg L^{-1})



CONCLUSIONS

The optimized method was considered satisfactory for analysing for CBZ in small amounts of invertebrate tissues since the sensitivity was appropriate and the matrix effect and the low recovery were corrected by the addition of the isotopically labelled internal standard. The procedure has the advantage to be simple and fast.

ACKNOWLEDGMENTS

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