# New Approaches to the Monitoring of Contaminants of Emerging Concern, Bifenthrin, Estrone, and Diclofenac, in Marine Environments

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## Outline

- Effects of contaminants in the environment
- Solid phase extraction methods
- Detection of bifenthrin and diclofenac in marine water
- New approaches to monitoring contaminants of emerging concern
- Sol-Gel chemistry
- Testing and initial results

## Solid Phase Extraction and Methods

Solid phase extraction (SPE) involves the isolation of analytes from a sample. The sample passes through a solid phase, where the analytes adsorb due to their affinity. A solvent is used as the mobile phase to elute and recover the analyte from the solid phase. After elution, the sample is dried and reconstituted in a smaller volume of mobile phase to increase the concentration for better detection during analysis. 100 mL of sample was spiked with a standard solution of the analyte with a concentration of 100 ppb. Oasis HLB 6cc 200 mg extraction cartridges were used for the SPE process. During SPE the sample was washed with ultrapure water and the analyte was eluted using 5 mL of acetonitrile. This was then evaporated to dryness using a steady stream of $N_2$ gas. The dried contaminants were then reconstituted in 1 mL of 20/80 (% v/v) methanol/water.

## HPLC-UV Detection of Diclofenac and Estrone

High-performance liquid chromatography was used to detect diclofenac and estrone (E1) with a UV detector, this can be seen in Figure 2.

HPLC-UV gradient conditions: 5 % B, 0 - 5min % B increased to 10%, 5 - 14 min B increased to 40 % B, 14-30 min B increased to 70%, from time 30.01-37 min B stayed at 100% B, %B was then returned to 5% for a re-equilibration time of 11 min, UV detector conditions: 275 nm.

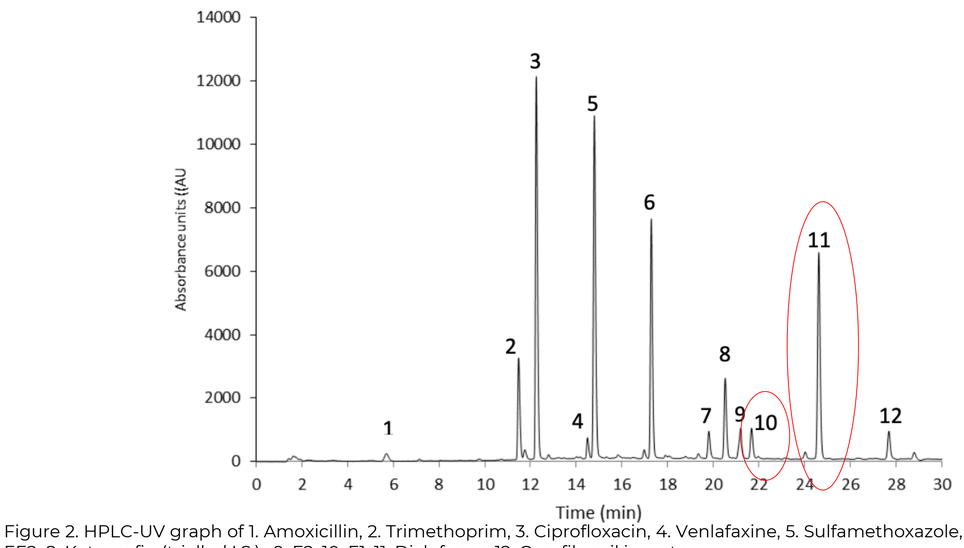
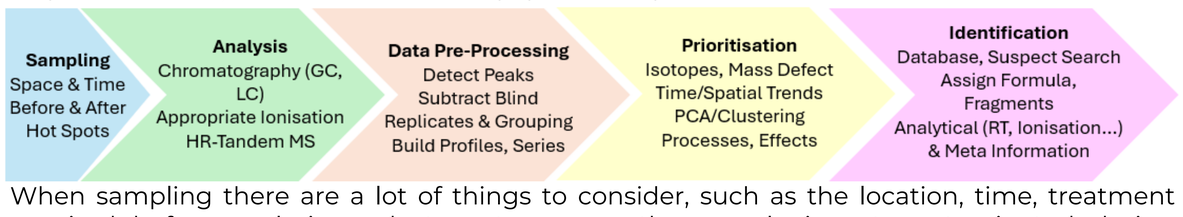



Figure 2. HPLC-UV graph of 1. Amoxicillin, 2. Trimethoprim, 3. Ciprofloxacin, 4. Venlafaxine, 5. Sulfamethoxazole, 6. Carbamazepine, 7. EE2, 8. Ketoprofin (trialled I.S.) , 9. E2, 10. E1, 11. Diclofenac, 12. Gemfibrozil in water

## The Need for New Approaches

The current methods of detection identify a need for new approaches due to the number of steps to consider, and the cost of the equipment required.




When sampling there are a lot of things to consider, such as the location, time, treatment required before analysis, and steps to ensure the sample is not contaminated during collection or transport. The analysis requires expensive equipment that has a lot of data pre-processing. After analysis there is a lot of data analysis required including prioritisation and identification. This could be solved by a real-time monitoring, selective sensor.

## Sol-Gel Chemistry and Process

Sol-gels are polymers with a silicon backbone. The silicon-based precursors are added to a glass jar with water, ethanol, and hydrochloric acid and is stirred at room temperature. This is spin coated on a substrate and dried in an oven at 120°C for 2 hours. The coated substrate is measured in a Fourier-transform infrared spectrometer (FTIR) as a blank. This is then submerged in a solution of analyte, rinsed with deionised water, and allowed to air dry before being measured in FTIR again to test for absorbance of analyte. This process is shown in Figure 7. The peaks indicate bonds present in the molecules present. This creates a fingerprint which can be compared to a standard of the analyte.

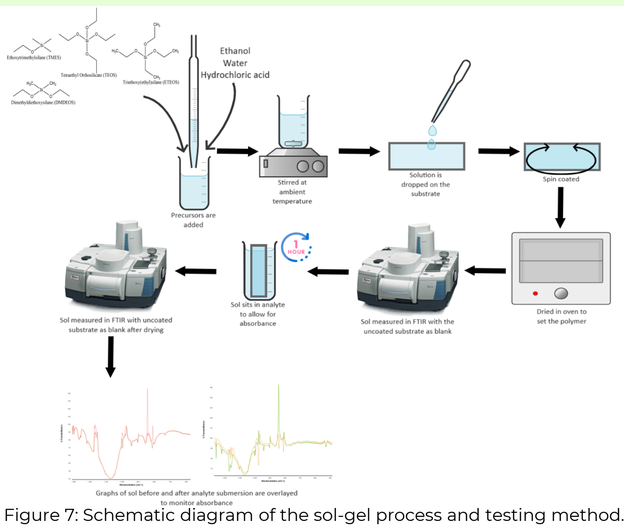



Figure 7: Schematic diagram of the sol-gel process and testing method.

The sol-gel for this initial analysis was prepared using three different silane precursors; tetraethyl orthosilicate (TEOS), diethoxydimethylsilane (DMDEOS), and triethoxy(ethyl)silane (ETEOS).

## Conclusions

- New materials can show promise for analyte enrichment or extraction
- Potential for easy-to-use optical sensors
- Potential for longer-term monitoring rather than a single grab sample

## References

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## Why Detect These Contaminants?

Pesticides, hormones, and pharmaceuticals are contaminants of emerging concern that can be found in many environmental samples, such as marine water. These can have many undesirable effects to the environment and public health.

Bifenthrin, shown in Figure 1 (A), is a pesticide, which easily binds to soil particles, causing residues to be carried into the environment. The toxicity of BF to non-target organisms is of most concern, highly toxic to fin-fish and crustaceans.[1,2] Low concentrations of bifenthrin have also been shown to cause behavioural effects in fish, raising concerns of neurotoxic properties in fish species, impacting the ability to forage and escape from predators.[3]

Diclofenac, Figure 1 (B), is a pharmaceutical used for anti-inflammatory and pain relief. This has a short half-life in seawater, 5 min – 11.55 days. However, due to the continuous input from wastewater treatment plants the concentration of diclofenac remains concerning in marine environments. Diclofenac is found to adsorb to suspended solids and sediments which can prevent it from breaking down. Exposure to diclofenac has been shown to affect egg development and has also show to affect the behaviour of adult Japanese rice fish.[4]

Estrone, Figure 1 (C), is a hormone which is of particular concern in aquatic environments due to its bioactive nature. Estrone can induce intersex and skew female : male ratios in fish. It can also increase male mortality and decrease reproductive success.[5]

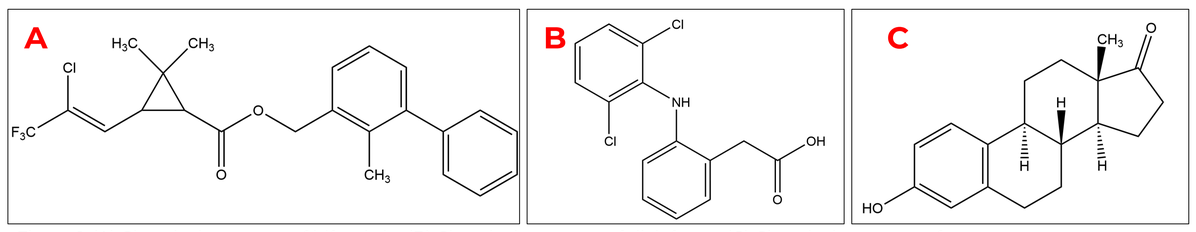


Figure 1: (A) Chemical structure of bifenthrin; (B) Chemical structure of diclofenac; (C) Chemical structure of estrone

## LC-MS Detection of Diclofenac

Liquid chromatography coupled with mass spectrometry (LC-MS) is a far more sensitive method of detection than HPLC-UV. Diclofenac was run in the LC-MS (QQQ) alone (Figure 3) and the fragments were preset to allow for the detection in a matrix. A matrix of pharmaceuticals including diclofenac was analysed and diclofenac was successfully detected (Figure 4).

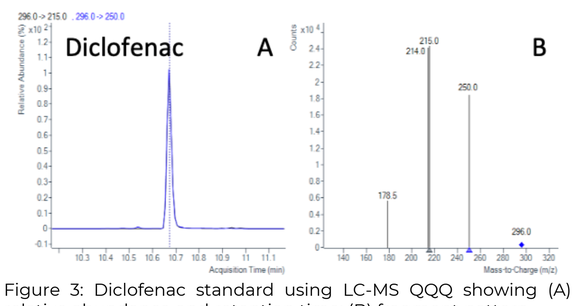


Figure 3: Diclofenac standard using LC-MS QQQ showing (A) relative abundance and retention time; (B) fragment pattern.

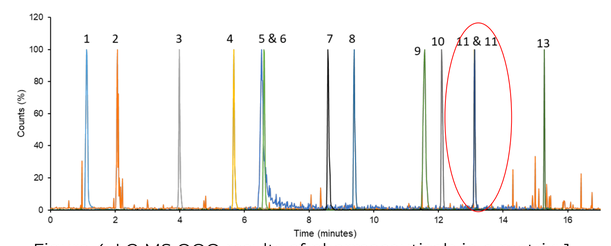


Figure 4: LC-MS QQQ results of pharmaceuticals in a matrix; 1. Metformin, 2. Amoxicillin, 3. Gabapentin, 4. Trimethoprim, 5. Ciprofloxacin, 6. O-desmethylvenlafaxine, 6. Venlafaxine, 7. Erythromycin, 8. Carbamazepine, 9. Azithromycin, 10. Clarithromycin, 11. Diclofenac, 13. Sulfamethoxazole

## LC-MS Detection of Bifenthrin

Bifenthrin was analysed using LC-MS QQQ using multiple reaction monitoring mode (MRM) (Figure 5). This allowed for the detection of bifenthrin at a concentration of 0.5 ppb in a river water matrix. Bifenthrin was also detected in a matrix of pesticides (Figure 6) also using the LC-MS QQQ.

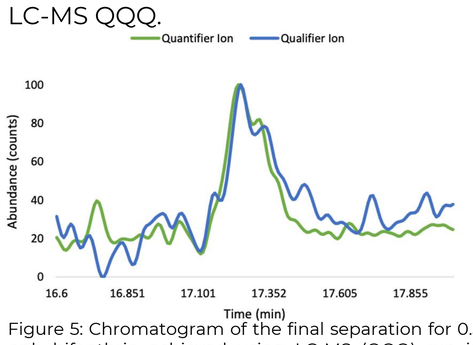


Figure 5: Chromatogram of the final separation for 0.5 ppb bifenthrin achieved using LC-MS (QQQ) run in MRM mode using a c18 2.1 X 150 mm, 1.9 µm column, 0.25 mL/min, 100 µl injection volume, gradient elution with mobile phase a) 0.1% FA + 5mm ammonium formate in Ultrapure Water b) 0.1% FA + 5mm ammonium formate in MeOH.

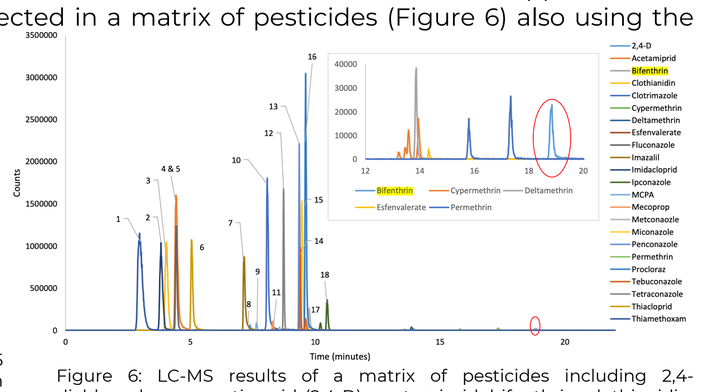


Figure 6: LC-MS results of a matrix of pesticides including 2,4-dichlorophenoxyacetic acid (2,4-D), acetamiprid, bifenthrin, clothianidin, clotrimazole, cypermethrin, deltamethrin, esfenvalerate, fluconazole, imazalil, ipconazole, 2-methyl-4-chlorophenoxyacetic acid (MCPA), mecoprop, metconazole, miconazole, penconazole, permethrin, prochloraz, tebuconazole, tetraconazole, thiacloprid, and thiamethoxam.

## FTIR Results

FTIR spectrum was taken of pure estrone in a solution of methanol, Figure 8, this was measured using salt plates with the clean salt plates as the background. Figure 9 shows the FTIR spectrum of the sol-gel after submersion in estrone, using the blank coated polymer as a background. Many peaks can be seen in the fingerprint region (1500-500 $cm^{-1}$). Peaks 1 and 2 are due to the $sp^3$ C-H bonds. These peaks are also observed in the spectrum of the sol-gel after submersion. Peak 3 corresponds to the alkoxyl C-O group. A large peak is also observed in this region of the sol-gel spectrum. Peaks 4 and 5 are due to the aromatic $sp^2$ bending in meta-di-substituted compounds.

The correlation between the peaks in the fingerprint region indicates that estrone was successfully absorbed in the sol-gel thin film. Further work will be carried out to increase selectivity of analytes.

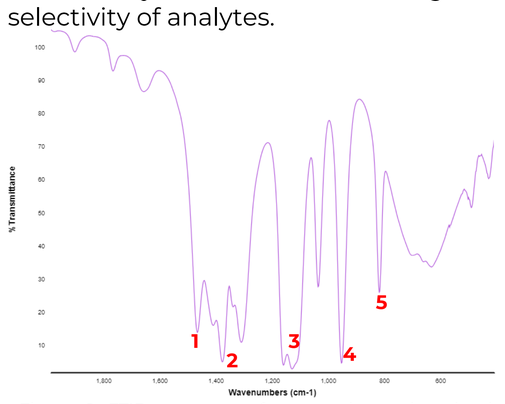


Figure 8: FTIR spectrum of estrone in methanol using salt plate, with the clean salt plates as the background.

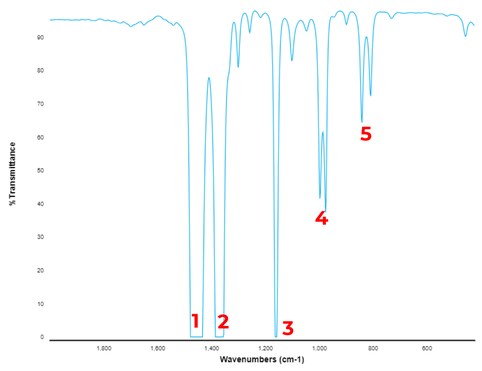


Figure 9: FTIR spectrum of the sol-gel coated polymer after submersion in estrone, using the coated polymer before submersion as the background

The research leading to these results has received funding from Horizon Europe, the European Union's Framework Programme for Research and Innovation (HEU 2021-2027) under grant agreement n° 101135796.