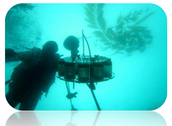
Enhancing Bioassay Directed Analysis Using Submersible Time Integrative SPE Multimedia Trains

AQUALYTICAL

C·I·Agent STORM·WATER SOLUTIONS

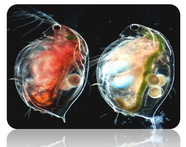
Collecting the Evidence for EDA



Effect Directed Analysis was developed where bioassay acts as the environmental "canary", to trigger instrumental analysis. The merging of ecotoxicology with analytical chemistry is responsible for finding ever emerging chemicals of concern.

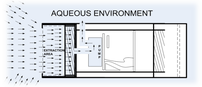
Analytical analysis of the organic fraction of the puzzle often requires large volumes of water to be extracted. The ideal extract for the EDA process should be time integrative that represents both total and dissolved **quantitative** fractions, of up to 100 liters of water. The extraction device must provide a clean blank, and the sequestering media a wide polarity of trace organics.

A solid phase extraction (SPE) device was envisioned that could provide this time integrative extract. It was necessary that this tool could provide submersible extractions unattended in the field , or on the bench. The device that was developed is explained below.

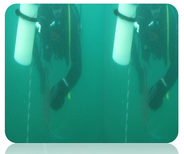


The Device and Method

A Submersible SPE Extractive Sampler was Designed to meet these Requirements.



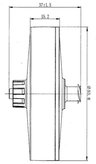
The **C.L.A.M.** (Continuous Low -Level Aquatic Monitoring) was developed to produce this unique extract. The **C.L.A.M.** is a small submersible extraction sampler, using EPA approved methodology 3535, utilizing SPE (Solid Phase Extraction) media to sequester Pesticides, Herbicides, PAH's, TPH, and other trace organics from water.



C.L.A.M.s weigh just over one pound, including the 4 AA batteries, and many can be easily taken to remote areas and left unattended to sample continuously for up to 36 hours at submerged depths up to 100 feet in marine or fresh waters.

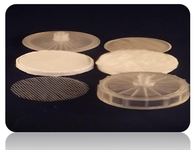
The **C.L.A.M.** actually extracts the water in-situ, with the same technology the labs use on the bench. It provides a pre-extracted quantitative sampling event, representing up to a hundred liters of water, lowering the laboratory detection limits a hundred fold. The small dry extraction disk is all that is sent to the laboratory for solvent elution and analysis

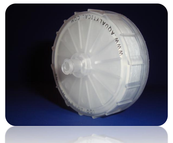
Media Disks Design and Capabilities



The C.L.A.M uses field hardened disks to actively draw a known volume of water through the SPE media during the extraction event. All the SPE media types are supported in custom housings which incorporate inlet flow dispersion, triple lofted glass pre-filters, supporting filters, and inert screens to field harden and reduce clogging.

All the Media disks are designed with Luer Lok ends which can connect one or many disks together, and Luer plugs can be used to seal the disks before and after deployment, for secure transport..





The SPE media disks encased in the CLAM disks are the same used by laboratories for the SPE 3535 extractions world wide. A large media selection is available as shown in the samples below;

•**C-18** for neutral pesticides , and neutral 8270 compounds

•**DVB** Used as the C-I8 media but will sequester compounds with greater polarity.

•**HLB** both Medium and Heavy polymer, is a solid-phase sample preparation product for the analysis of EPA Method 8270 (semi-volatile organics), EPA Method 1694 (pharmaceuticals and personal care products), endocrine disruptors, and other emerging contaminants in wastewater and drinking water

• **8270 One Pass Disk,**(combining HLB, Cationic and Anionic phases to extract neutrals, acids and bases from the water requiring a mullti step elution scheme.

• **Activated Carbon Disk,** Sequesters very polar and or volatile organics, often run in extraction trains, where the disks can be Luer Locked together in any arrangement.

•**Pre-filter Disks,** Developed for obtaining both total and dissolved trace organic fractions. Used in field extractions submerged in situ or on the bench. The disks can be Luer locked together to form a 2,3,or 4 disk extraction train. sequestering totals, dissolved, non-polars, and polars or breakthrough.

University Enhancing Toxicological Studies with SPE Disk Extraction Train

Duke UNIVERSITY

NICHOLAS SCHOOL OF THE ENVIRONMENT DUKE UNIVERSITY *forging a sustainable future*

Study Overview

14 day study 5 Sites

•Three wastewater impacted storm water retention ponds
•One WWTP lagoon
•One dechlorinated municipal water (Charleston County) as a control.

. Toxicology

•Male adult fathead minnows.
analysis: sample tissues (liver, gonad, brain) were analyzed using a non-gel based, label free MS based method to identify differentially expressed proteins among treatment groups.

Chemistry Sampling

• CLAM discs (Oasis HLB) 24 hour continuous sampling 7 days total
• Disk extraction Train: Lofted pre-filter, HLB disk, HLB Breakthrough disk.
•Automated SPE (Dionex Autotrace 280) 7 days total
•Grab samples for large volume direct injection analysis14 days
I

Extraction and Laboratory Analysis

Discs were preconditioned, spiked, & deployed for 24 hours, sampling continuously through the media with a peristaltic pump at a rate of 3-4 mL/min. A prefilter disc was used ahead of the CLAM series to prevent particulate buildup in the CLAM discs that would disrupt peristaltic flow (pond water is laden with algae and other NOM). Two discs were used in series to detect possible breakthrough of analytes from the first disc

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Laboratory Extraction and Analysis

Solvent elution of field extracted disks, follow EPA method 3535 for SPE elution procedures.. The elutant simply has to be made compatible to the instrument type. In this event the Discs were eluted with 20 ml of MeOH/MTBE (10/90) and dried with sodium sulfate. The extracted was blown down with N2 to near dryness and reconstituted to LC mobile phase (25%ACN,75% H2O).

Organic micro contaminants were quantified in extracts by LC-UHPLC with high resolution mass spectrometry (ThermoFisher LTQ-Orbitrap Velos mass spectrometer). Chromatography on a 100mm x 2.1mm (1u particle size) C-18 Hypersol Gold column by Thermo. A 25 uL full loop injection with linear acetonitrile/water gradient with post column addition of 100 mM NH4OH was used with detection by ESI (+) and (-) with quantitation by full scan high resolution mass spectrometry. A 6 point calibration curve was generated daily that spanned 5 orders of magnitude (with R^2>.999)

Resulting Extraction Data

Compound	Average Water Concentration (ng/l) ND = <1				
	Pond #5	Pond #25	Pond #43	WWTP	Control
hydroxychlorothalonil	ND	ND	ND	ND	ND
Azoxystrobin	13.8	23.9	186.7	16.3	ND
Metalaxyl	ND	ND	ND	ND	ND
Fenarimol (Bloc)	ND	ND	ND	ND	ND
Iprodione*	ND	ND	ND	ND	ND
Flutolanil	ND	ND	ND	ND	ND
Propiconazole	ND	1.9	67.0	2.3	ND
Fipronil	ND	ND	1.7	13.5	ND
Fipronil-desulfinyl	ND	1.3	9.1	12.1	ND
Fipronil-sulfone	ND	2.5	13.6	24.5	ND
Fipronil-sulfide	ND	ND	6.2	34.5	ND
Fenamiphos	ND	ND	ND	ND	ND
Atrazine*	38.7	277.5	239.1	58.5	ND
Atrazine-desisopropyl	<1	ND	ND	ND	ND
Atrazine-desethyl	21.5	95.9	94.6	75.8	ND
Bromoxynil*	ND	ND	ND	ND	ND
Oxadiazon	ND	ND	16.2	ND	ND
Quinclorac	ND	ND	ND	ND	ND
Pronamide	ND	ND	ND	ND	ND
Mecoprop (MCPP)	ND	ND	ND	ND	ND
2,4-D*	ND	ND	ND	ND	ND
Bisphenol A*	3.6	8.4	18.1	63.2	5.0
a-Ethynylestradiol*	ND	ND	ND	ND	ND
b-Estradiol*	ND	ND	ND	ND	ND
Estrone*	ND	3.6	14.7	34.4	ND
Estriol*	ND	ND	ND	ND	ND
Genistein*	ND	ND	ND	3.0	ND

Recovery of spiked Analytes on the Extraction Train

Octanol - Water coefficients determined if the field spiked surrogates were adsorbed or sequestered by the glass prefilter or the media. Water soluble analytes or polars were retained by the HLB media, and non-polars were retained on the glass pre-filter proportional to the KOW value.

Recovery of spiked analytes from CLAM disks

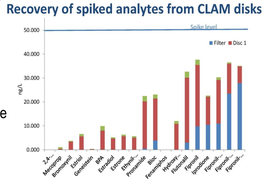

Figure 4. Variable recovery was observed for analytes spiked (at 50 ng/l) in 50 mL of nonopure water after extraction using CLAM disks. Little breakthrough was observed onto a second CLAM disk, but several analytes were strongly retained by the glass fiber CLAM prefilters.

Conclusions

Significant concentrations of turf grass management chemicals were detected in storm water retention ponds on Kiawah Island, SC – presumably due to runoff from residential use and fairway maintenance operations.

• CLAM Disks are convenient, effective devices for integrative sampling of organic micro contaminants in water and wastewater environments at part-per-trillion levels.

• CLAM devices hold great promise for quantitative analysis of ambient micropollutant concentrations in water to support toxicological assessments.

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