Comparison of Automated and Manual Solid Phase Extraction (SPE) for Contaminants of Emerging Concern (CECs) Analysis

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Outline

- Background
- Endocrine disruptors
- Testing method
- Sample preparation
- SPE process
- Cartridge
- Data
- Recommendations

Background

- **CECs refer to any chemical discovered in water or in the environment that had not previously been detected, or**
- **only present at insignificant levels.**
- **CECs range from pharmaceuticals and personal care products (PPCPs) to persistent organic pollutants used in many industrial processes**
  - **Disinfection by-products**
  - **Endocrine disruptors (EDCs)**
  - **Industrial Chemicals**
  - **Natural Toxin Chemicals**
  - **Persistent Organic Pollutants (POPs)**
  - **Pesticides**
  - **Pharmaceutical and Personal Care Products (PPCPs)**

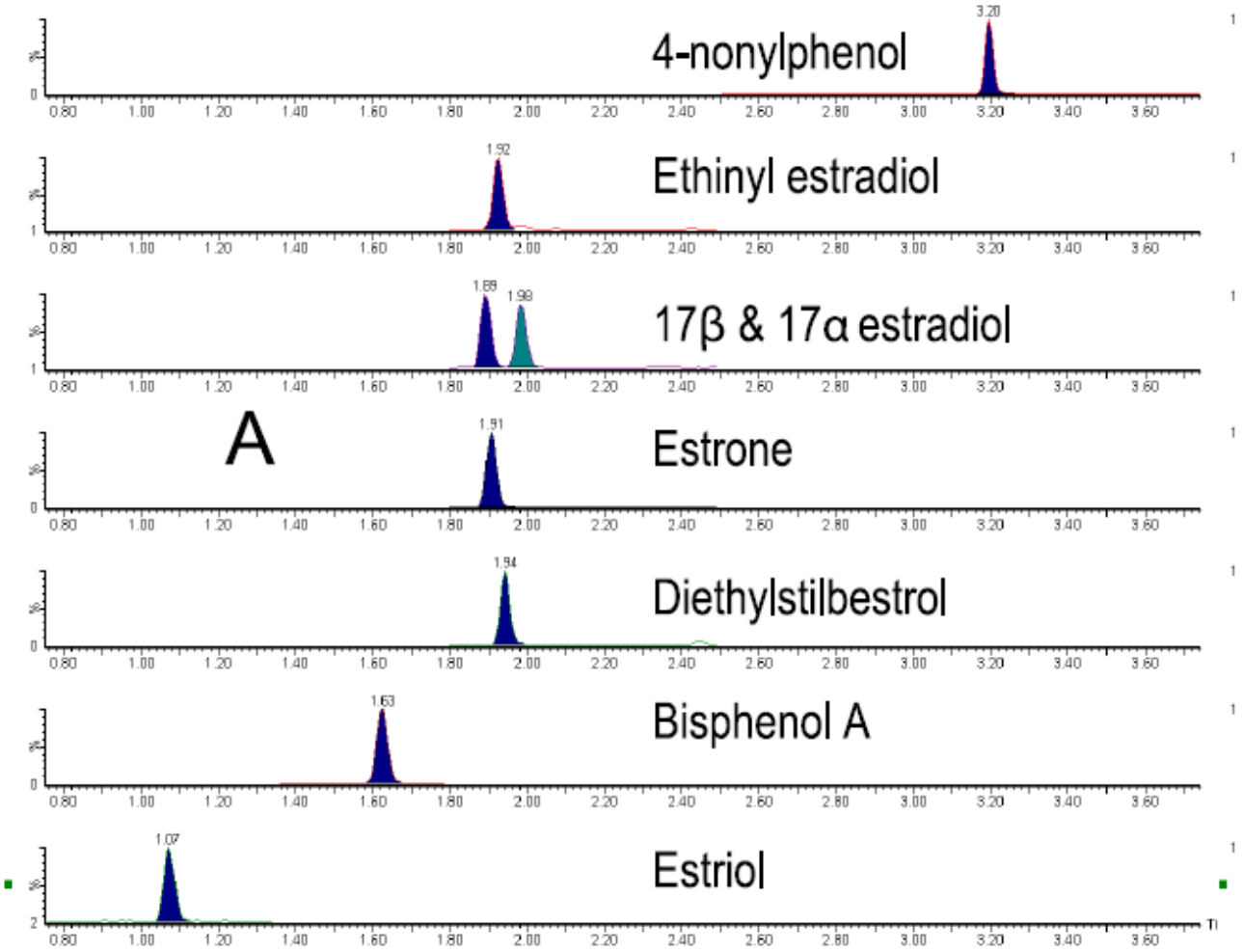
Endocrine disruptors (EDCs)

- EDCs can alter the normal functions of hormones
- Health effects: developmental, reproductive, neurological, or immune deficiencies in humans, animals, aquatic organisms
- EDCs:
  - Pesticides
  - pharmaceutical products
  - Plasticizers
  - Dioxins
  - Hormones
  - Bisphenol A leached from metal cans
- cause significant reproductive effects at very low levels

Testing Method

GC/MS, LC/MS are beneficial tool for monitoring CECs in environmental samples:

- Ability to analyze a wider range of compounds in a single analysis
- Unmatched specificity to ensure reliability and accuracy



Source: Waters

- Sensitivity to detect very low levels of toxins
- Enhanced confidence through use of library databases
- Utilize unknown screening approaches to identify unknown or emerging PPCP compound or possible metabolites
- Require sample extraction procedures

LC separation

- **Instrument:** Agilent 1200 HPLC system
- **Column:** Xselect CSH™ C18 HPLC column (130 Å pore size, 3.5 µm particle size, 2.1 mm i.d. × 150 mm length) from Waters
- **Inject volume:** 10 µL
- **Flow rate:** 200 µL/min
- **Gradient elution:**

Time (min)	Water/methanol (96:4, v/v), 5mM NH_4OH	Water/methanol/acetonitrile (10:10:80, v/v/v), 5mM NH_4OH
2	100%	0%
5	70%	30%
7	70%	30%
17	0%	100%
25	0%	100%
26	100%	0%
35	100%	0%

MS detection

- **Instrument:** TSQ Quantum™ Ultra Triple Quadrupole Mass Spectrometer
- **Mode:** ESI MRM mode, perform ESI (+) and ESI (-) in single EZ method
- **Spray voltage:** 4000 V (positive mode); 2500 V (negative mode)
- **Capillary temperature:** 350 °C
- **Sheath gas pressure:** 35 Arb
- **Aux gas pressure:** 30 Arb

Source: U of B

GC-MS/MS method

GC separation

- **Instrument:** Thermo Scientific Trace GC Ultra
- **Column:** DB-5HT column, 30 m × 0.25 mm i.d. × 0.1 µm film thickness
- **Carrier gas:** Helium, 1 mL/min
- **Injection type:** Splitless
- **Inlet temperature:** 67 °C
- **Inject volume:** 1.5 µL
- **Oven program:**

Temperature (°C)	Hold time (min)	Rate (°C/min)
60	1	0
160	2	15
190	0	2.2
290	3	50
330	2	15

MS detection

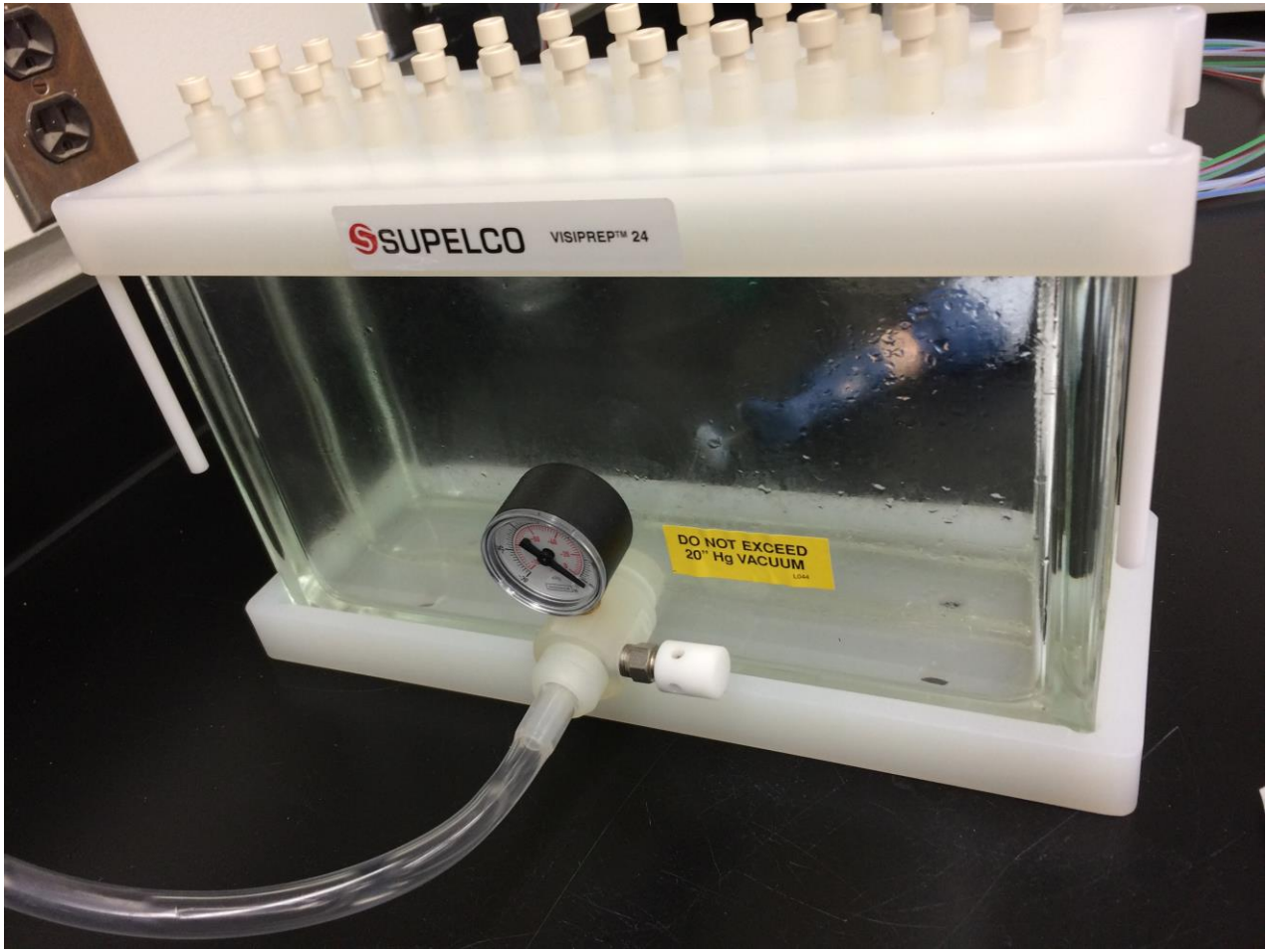
- **Instrument:** TSQ Quantum XLS mass spectrometer
- **Mode:** EI positive MRM mode (70 ev)
- **Ion Source Temperature:** 350 °C

Source: U of B

Sample Preparation

- Manual SPE
- Visiprep™ SPE Vacuum Manifold, standard 24-port model
- Sigma-Aldrich, list price $1610.00
- Spacing between ports are too small to allow simultaneous samples process
- Control of flow rate is critical

Visiprep SPE



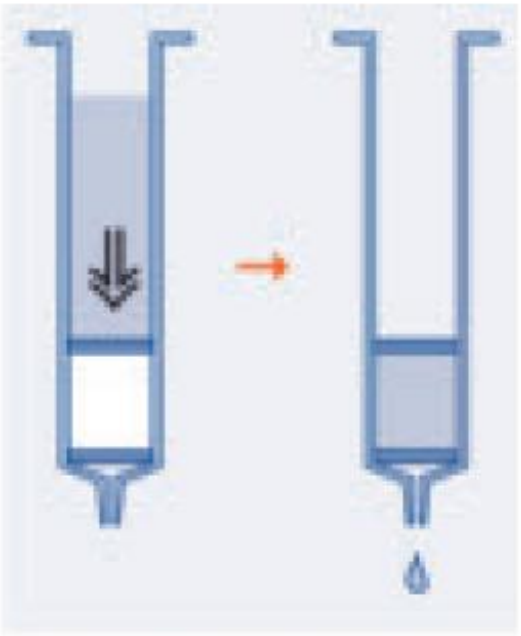
Sample Preparation

Sample Preparation

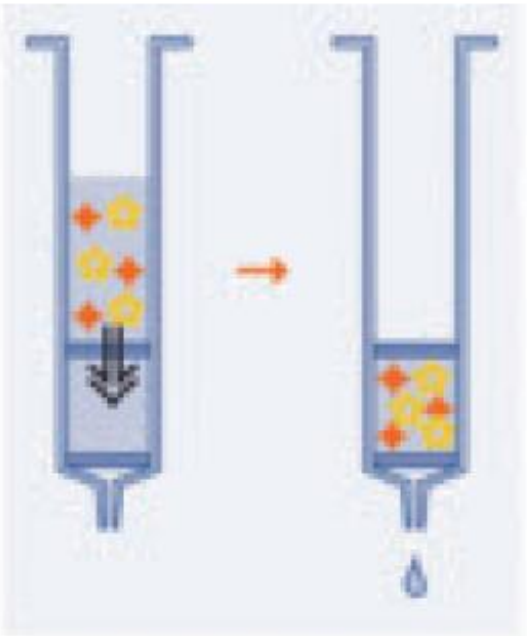
- Automated SPE
- Fast sample preparation
- Ease of automation
- Reduction in organic solvent consumption
- Enhanced laboratory productivity and reduced costs
    - Unattended operation
    - Batch processing of up to 6 samples
    - Reduce solvent usage
    - Segregates aqueous and solvent waste using separate waste lines for efficient, cost-effective waste handling
- Flexibility in operation
    - Loads sample volumes from 20 mL to 4 L
    - Compatible with 1, 3, and 6 mL cartridges and with 47 mm SPE disks
    - Automatical conditions, rinses, and elutes SPE cartridges with a choice of five solvents
    - Offers a choice of six different collection vial racks

Sample Preparation

SPE Process



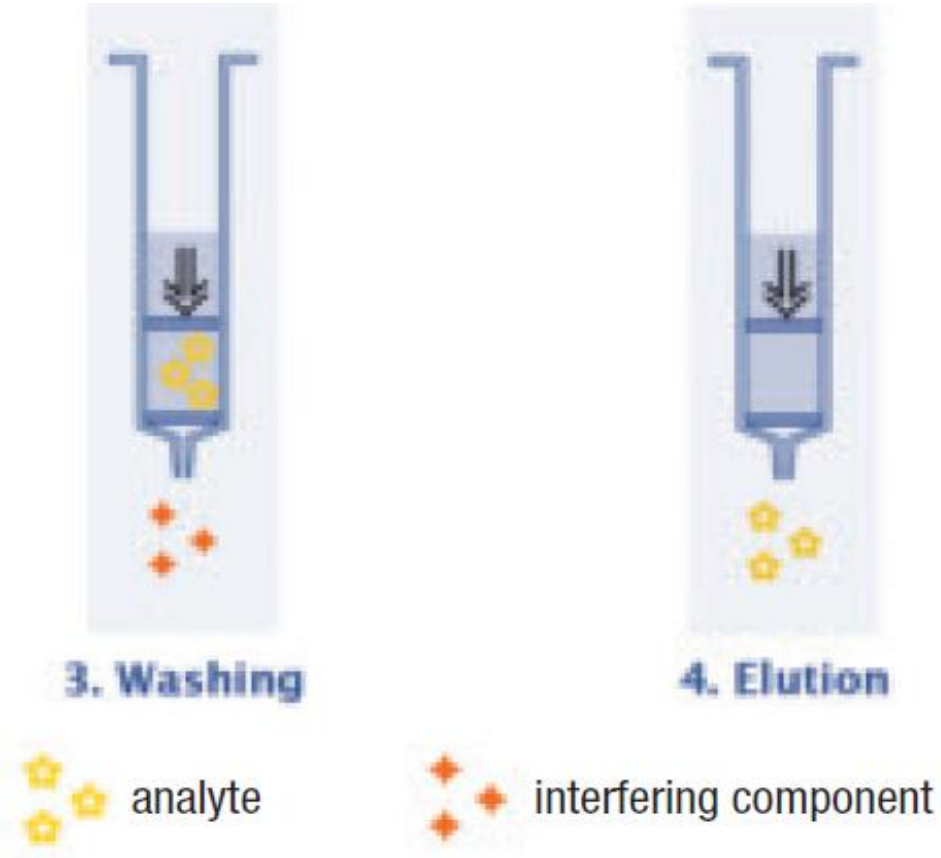
1. Conditioning



2. Sample application

Source: www.hichrom.co.uk

SPE Process



Source: www.hichrom.co.uk

SPE Mechanisms

- Normal Phase
  - Traditional silica based sorbents – effective for non-polar analytes
- Reverse Phase
  - Polymeric sorbents – higher capacity
  - **Oasis HLB** more effective for polar analytes and metabolites
- Ion Exchange
  - electrostatic interaction

REVERSE-PHASE	**Polymeric phases** based on Styrene-divinylbenzene Hydrophylic-Lipophilic Balanced phases (Oasis HLB) N-vinyl pirrolidone (Strata-X, Phenomenex) Bond-Elut Plexa (Agilent Technologies) In-house polymeric phases: PANI, PPy, 4-VP-DVB(4-vinylpyridine-divinylbenzene), 4-VIm-DVB, HXLPP (Hypercross linked polymeric sorbent) polar B [36]; cross-linked polystyrene divinylbenzene (SDB-XC (poly(styrenedivinylbenzene)) and SDB-RPS (poly(styrenedivinylbenzene) [57] **Silica-bonded phases** Octadecyl-bonded silica Octyl-bonded silica Butyl-dimethyl bonded silica Phenyl-bonded silica Hisep. Hydrophobic surface enclaved by a hydrophilic network
NORMAL-PHASE	Ciano-bonded silica Diol-bonded silica Graphene Oxide (GO) [58] Amino-bonded silica
ION-EXCHANGE	LC-SAX Quaternary Amine bonded silica with Cl- counterion LC-SCX Sulfonic acid bonded silica with Na^+ counterion [59] LC-WCX Carboxylic acid bonded silica with Na^+ counterion
MIXED-MODE SPE (ION-EXCHANGE+ REVERSED-PHASE)	**Polymeric structures** Polymeric strong cation-exchange — Polymer structure modified by sulfonic acid Polymeric weak cation-exchange — Polymer structure modified by carboxylic acid [60] Polymeric strong anion-exchange — Polymer structure modified by quaternary amine Polymeric weak anion-exchange — Polymer structure modified by secondary amine
ADSORPTION	Silica gel without bonded phase **Alumina-Based Packing – Crystalline, chromatographic grade alumina, irregular particles, 60/325 mesh** Alumina-A Alumina-B Alumina-C **Florisil®-Based Packing–Magnesium silicate, 100/120 mesh particles** LC-Florisil Envi-Florisil **Resin-Based Packing – 80–160 μm spherical particles** Envi-Carb P **Graphitized Carbon-Based Packing – Nonbonded carbon phase** Envi-Carb Envi-Carb C
SIZE EXCLUSION+ADSORPTION **ADSORPTION+MAGNETIC SPE**	**Graphitic Carbon Nitride (g-C_3N_4)** [61] **Restrict Access Materials (RAMs)** **g-C_3N_4/Fe_3O_4** composite materials [62]
MAGNETIC SOLID-PHASE EXTRACTION	**Graphene** [63] **Graphene/Fe_3O_4@polythiophene nanocomposite** [64] **Graphene oxide (GO)/Fe_3O_4 nano-hybrid** [65] **Fe_3O_4/SiO_2/P(MAA-co-EGDMA)** [33] **Conducting Polymers (CPs)** — **Polytiophene** **Polytiophene coated with Fe_3O_4**
ELECTROSTATIC DRIVING FORCE	**Magnetic NPs coated with MIPs** **Molecularly Imprinted Polymers**
IMMUNOSORBENTS (IMSs)	Based on molecular recognition by antibodies [66]
IONIC LIQUIDS	Immobilized onto a silica or polymeric surface [67]

Source: Víctor Cerdà et. al

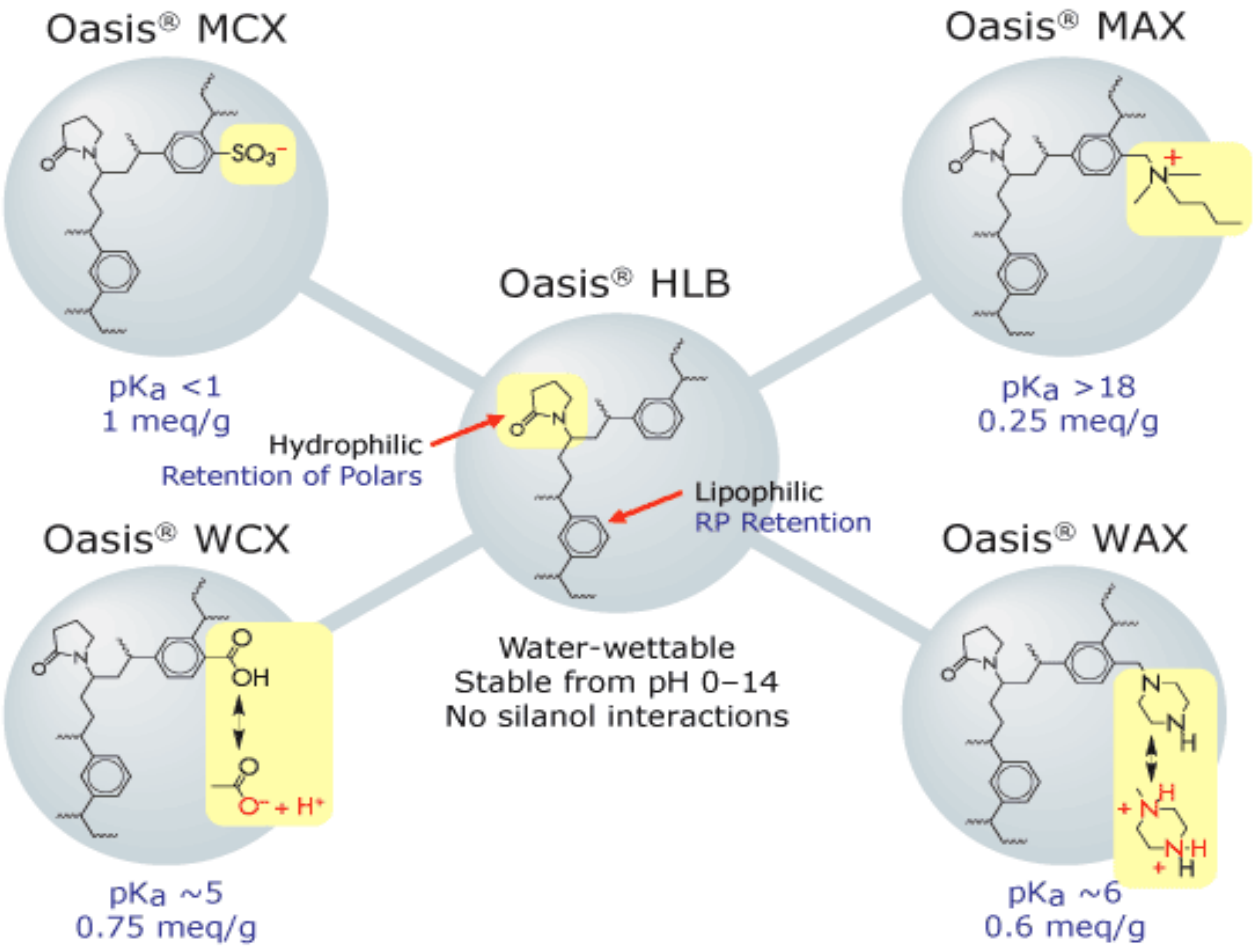
Cartridge: Oasis HLB

- Macroporous copolymer
- Consisting of a balance ratio of two monomer components:
    - lipophilic DVB (divinylbenzenevinylpyrrolidone) and hydrophilic
    - N-vinylpyrrolidone

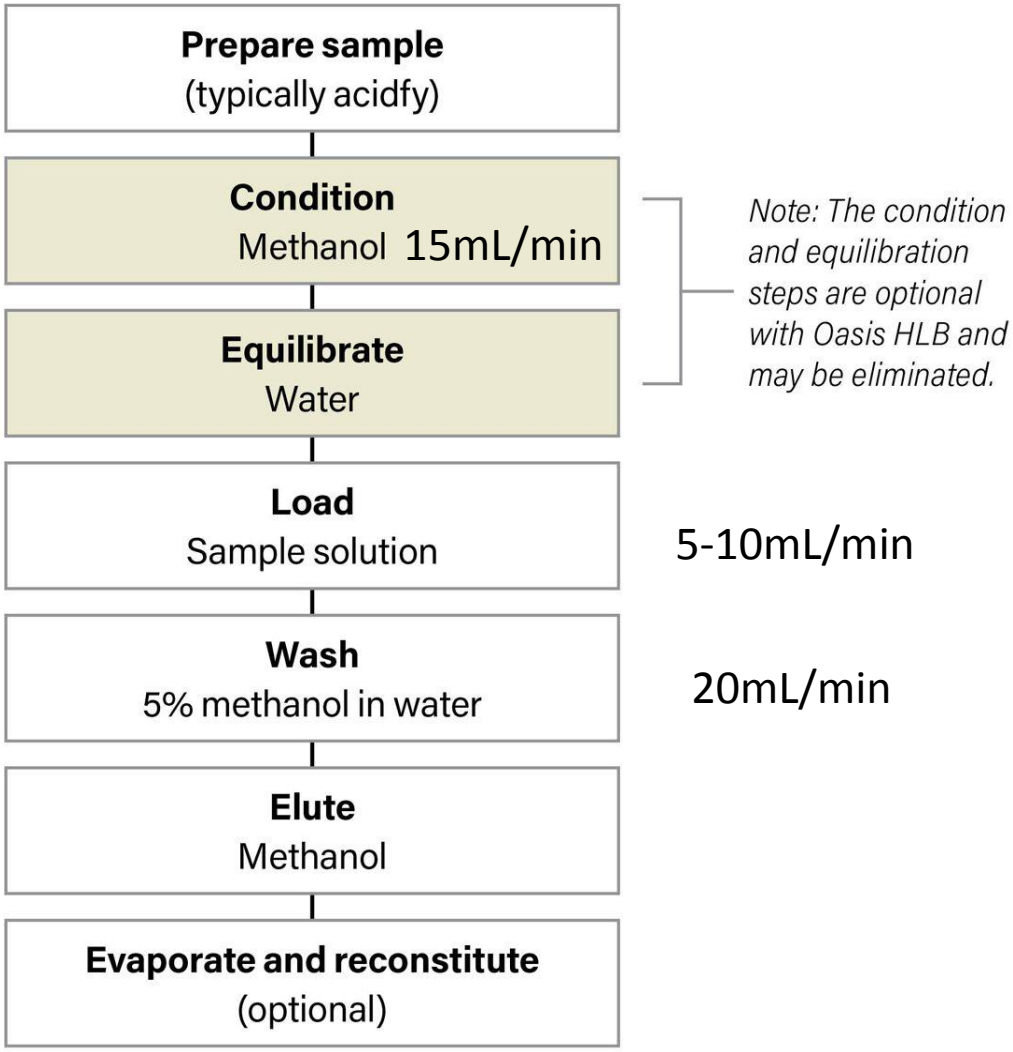
Cartridge: Oasis HLB

- good wettability to the resin and high mass transfer rates from aqueous solutions
- designed for sorptive preconcentration of organic compounds with a broad spectrum of polarities
- ideal for acidic, basic and neutral analytes, stable over the entire range of pH 0-14
- suitable for the extraction/clean-up of pharmaceuticals, personal care products, and hormones in a number of matrices, such as soils, sediments, and sludge

Oasis SPE Sorbents



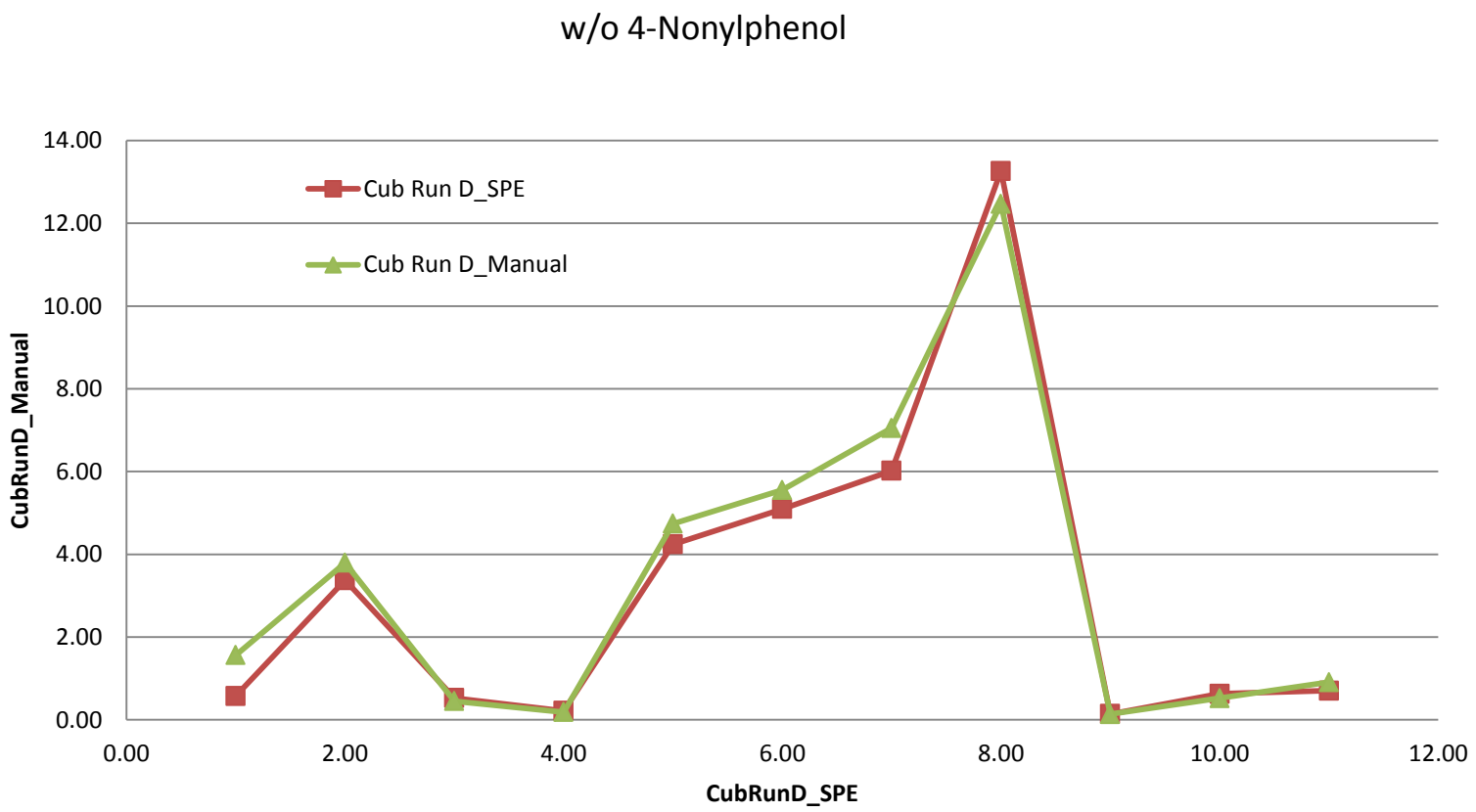
GENERIC METHOD OASIS HLB SPE



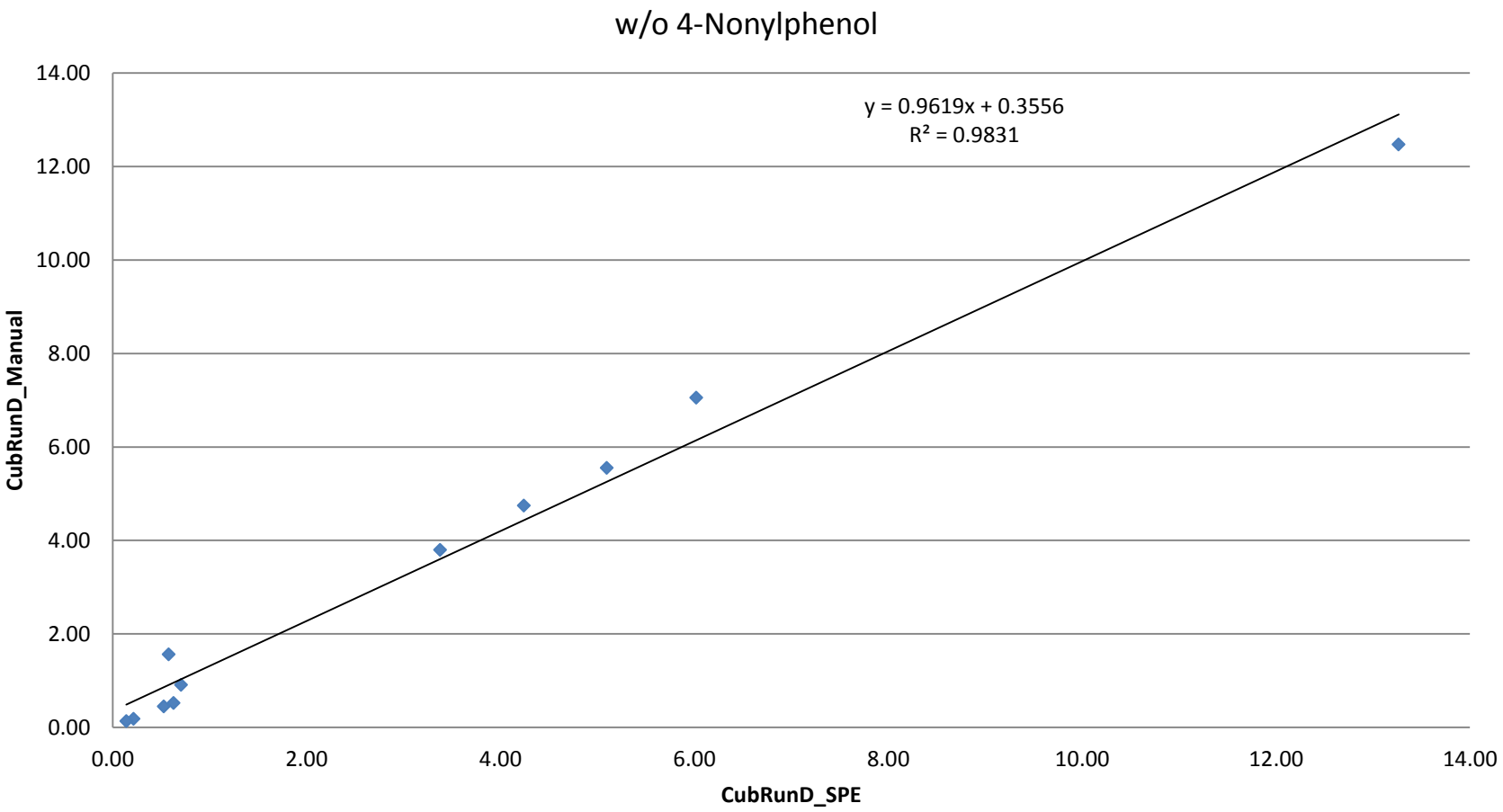
	Targeted analytes	Compound class
1	Estrone (E1)	Free Estrogen
2	17α-estradiol (E2α)	Free Estrogen
3	17β-estradiol (E2β)	Free Estrogen
4	17β-Estriol (E3)	Free Estrogen
5	17α-Ethinylestradiol (EE2)	Free Estrogen
6	estrone-3-sulfate (E1-3S)	Conjugated Estrogen
7	estrone-3-glucuronide (E1-3G)	Conjugated Estrogen
8	17β-estradiol -17-sulfate (β-E2-17S)	Conjugated Estrogen
9	17α-estradiol -3-sulfate (α-E2-3S)	Conjugated Estrogen
10	17β-estradiol -3-sulfate (β-E2-3S)	Conjugated Estrogen
11	17β-estradiol-3-glucuronide(β-E2-3G)	Conjugated Estrogen
12	17α-ethinylestradiol-3-glucuronide (EE2-3G)	Conjugated Estrogen
13	4-tert-octylphenol	Industrial compound
14	4-n-nonylphenol	Industrial compound
15	bisphenol A (BPA)	Industrial compound
16	Imidacloprid	Pesticide, neonicotinoid
17	Acetamiprid	Pesticide, neonicotinoid
18	Clothianidin	Pesticide, neonicotinoid
19	Dinotefuran	Pesticide, neonicotinoid
20	Fipronil	Pesticide, phenylpyrazole
21	Cycloate	Pesticide, thiocarbamate
22	Atrazine	Pesticide, triazine
23	Simazine	Pesticide, triazine
24	Prometon	Pesticide, triazine
25	Alachlor	Pesticide, chloroacetamide
26	Metolachlor	Pesticide, chloroacetamide
27	Acetochlor	Pesticide, chloroacetamide
28	Dichlorvos	Pesticide, Organophosphorus
29	Ethoprop	Pesticide, Organophosphorus
30	Tributylphosphorotrithioite	Pesticide, Organophosphorus
31	dimethoate	Pesticide, Organophosphorus
32	Diazinon	Pesticide, Organophosphorus
33	Chlorpyrifos	Pesticide, Organophosphorus
34	Coumaphos	Pesticide, Organophosphorus
35	Bromacil	Pesticide, substituted uracils

Source: University of Buffalo

Manual and SPE



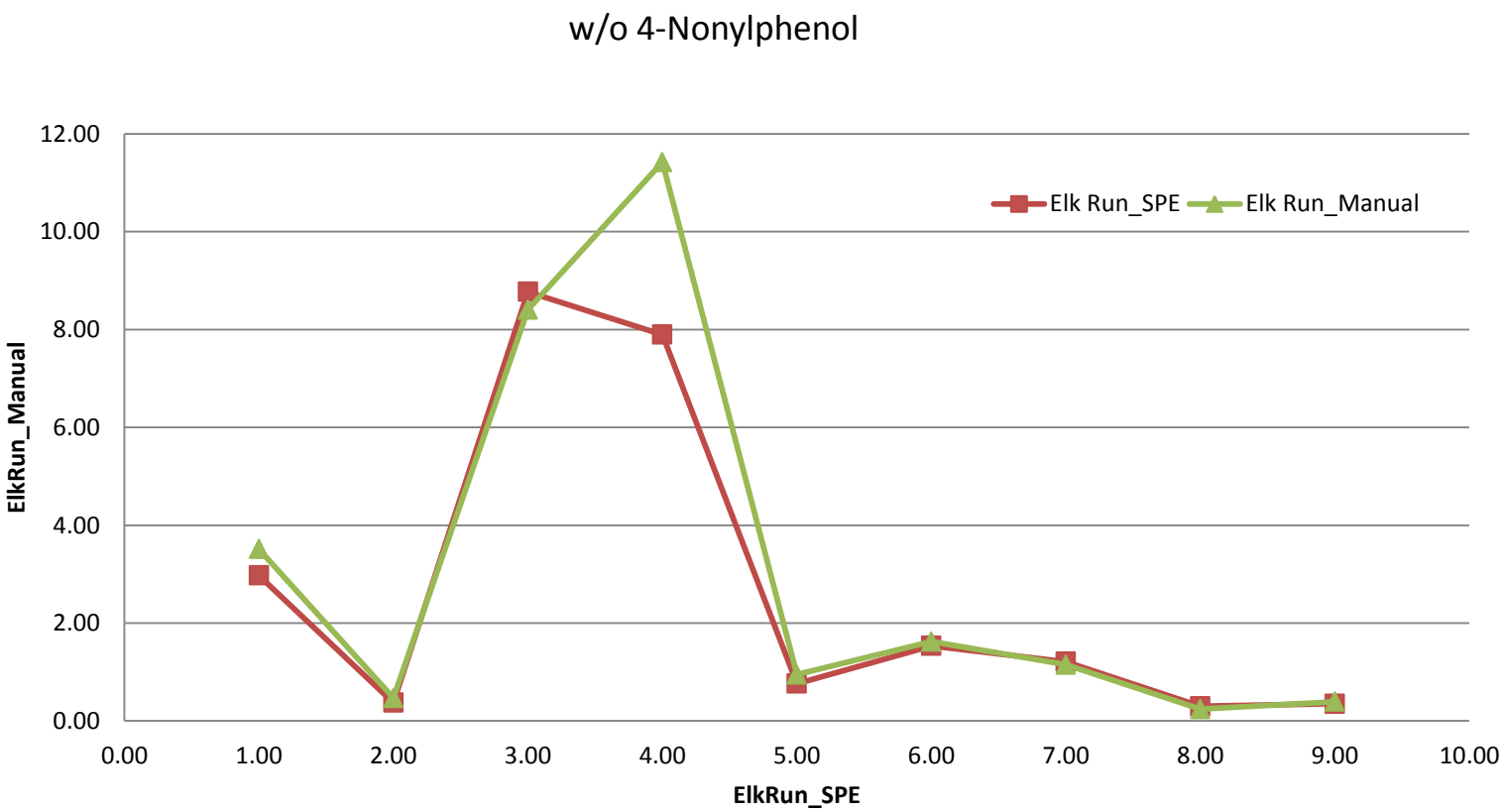
Manual and SPE



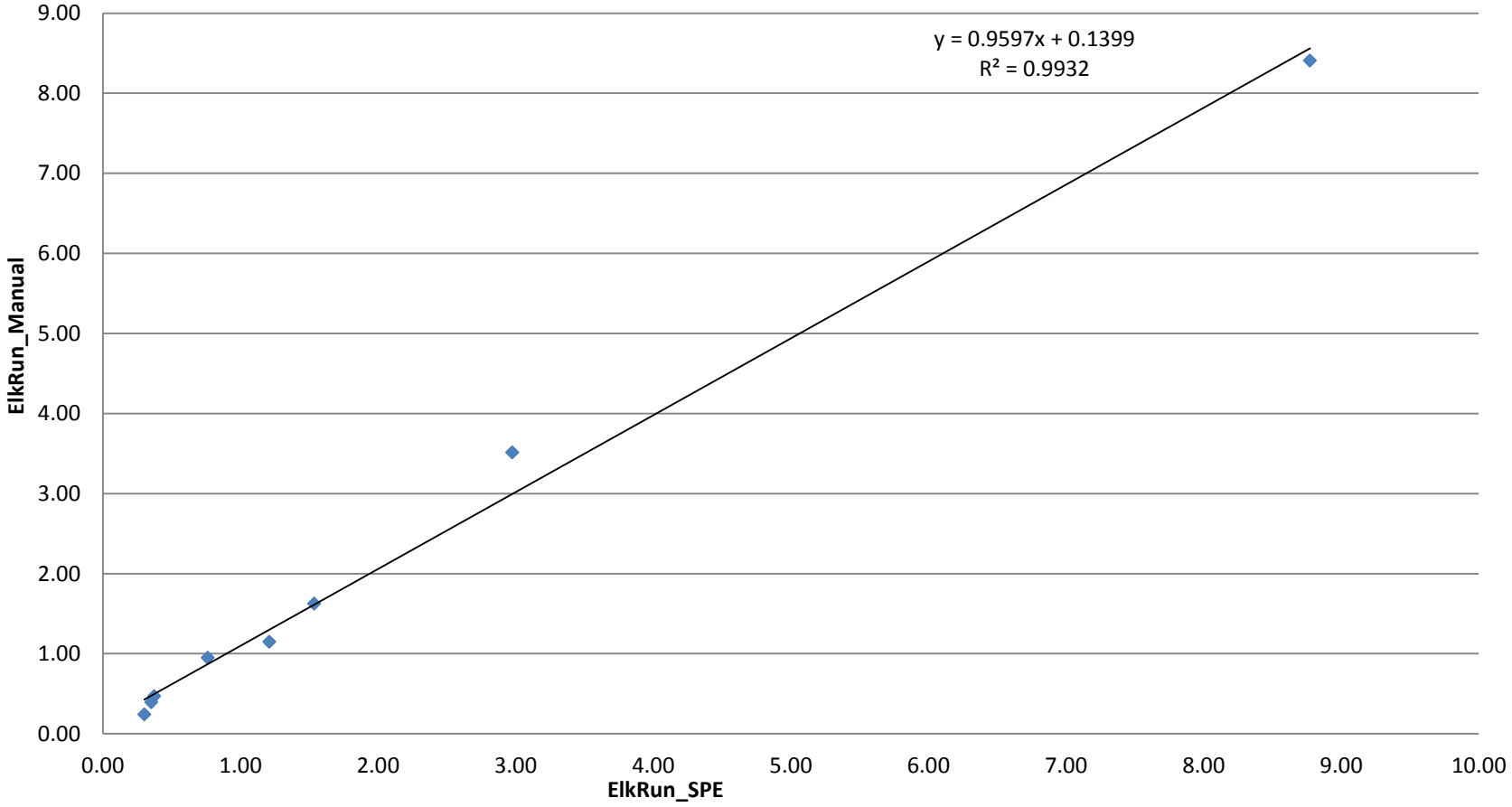
Outlier: 4-nonylphenol

	Cub Run_SPE	Cub Run_Manual	% difference
4-Nonylphenol	53.49	212.82	119.66
4-tert-octylphenol	0.58	1.56	92.09
Acetamiprid*	3.38	3.79	11.67
Acetochlor	0.53	0.45	15.69
Atrazine	0.22	0.19	15.19
Clothianidin*	4.24	4.74	11.19
Dinotefuran	5.10	5.55	8.58
Fipronil*	6.02	7.05	15.82
Imidacloprid	13.26	12.47	6.19
Metolachlor	0.14	0.13	3.21
Prometon	0.63	0.52	18.31
Simazine*	0.70	0.91	25.35

Elk_run


w/o 4-Nonylphenol
12.00
10.00
8.00
6.00
4.00
2.00
0.00
ElkRun_Manual
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
ElkRun_SPE
Elk Run_SPE
Elk Run_Manual

Elk_run


y = 0.9597x + 0.1399
R² = 0.9932
ElkRun_Manual
ElkRun_SPE
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00

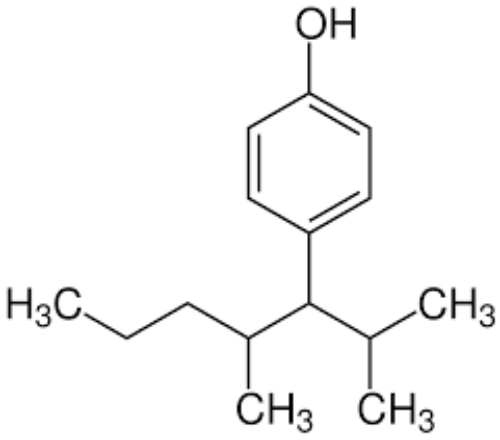
Outlier: 4-nonylphenol

	Cub Run_SPE	Cub Run_Manual	% difference	Elk Run_SPE	Elk Run_Manual	% difference	mm, g/mol	d, g/cm^3	Polarity
4-Nonylphenol	53.49	212.82	119.66	32.96	364.71	166.85	220.35	0.95	non-polar
Acetamiprid*	3.38	3.79	11.67	2.98	3.51	16.57	222.68	1.17	weak-polar
Acetochlor	0.53	0.45	15.69	0.37	0.47	22.69	269.77	1.10	weak polar
Atrazine	0.22	0.19	15.19	8.77	8.41	4.26	215.68	1.19	polar
Clothianidin*	4.24	4.74	11.19	7.90	11.42	36.49	249.68	1.61	high-polar
Fipronil*	6.02	7.05	15.82	0.76	0.95	21.80	437.15	1.48	polar
Imidacloprid	13.26	12.47	6.19	1.53	1.62	5.59	255.66	1.60	weak-polar
Metolachlor	0.14	0.13	3.21	1.21	1.15	5.21	283.80	1.10	polar
Prometon	0.63	0.52	18.31	0.30	0.24	22.25	225.29	1.10	polar
Simazine*	0.70	0.91	25.35	0.35	0.39	11.29	201.66	1.30	polar

Duplicate of SPE

February, 2018						
ng/L in water	UOSA	UOSA-Duplicate	% difference	mm, g/mol	d, g/cm^3	Polarity
4-Nonylphenol*	63.45	84.80	28.80	220.35	0.95	non-polar
Acetamiprid*	4.25	4.44	4.31	222.68	1.17	weak-polar
Atrazine	5.78	4.58	23.17	215.68	1.19	polar
Clothianidin*	44.72	5.81	153.99	249.68	1.61	high-polar
Dinotefuran*	19.63	22.27	12.57	202.21	1.40	polar
Fipronil*	0.47	0.60	24.21	437.15	1.48	weak-polar
Imidacloprid	37.38	33.70	10.36	255.66	1.60	polar
Metolachlor	1.68	1.79	6.17	283.80	1.10	polar
Prometon	1.10	1.23	10.44	225.29	1.10	polar
Simazine*	3.88	4.51	15.13	201.66	1.30	polar
March, 2018						
ng/L in water	UOSA	UOSA-duplicate	% difference	mm, g/mol	d, g/cm^3	Polarity
4-Nonylphenol*	96.43	160.98	50.15	220.35	0.95	non-polar
Acetamiprid*	1.22	1.10	9.95	222.68	1.17	weak-polar
Atrazine	10.74	9.82	8.95	215.68	1.19	polar
Clothianidin*	2.74	2.70	1.72	249.68	1.61	high-polar
Dinotefuran*	22.77	22.37	1.78	202.21	1.40	polar
Fipronil*	0.65	0.47	31.92	437.15	1.48	weak-polar
Imidacloprid	35.63	37.09	4.02	255.66	1.60	polar
Metolachlor	4.64	5.29	13.07	283.80	1.10	polar
Prometon	2.67	2.40	10.65	225.29	1.10	polar
Simazine*	2.86	2.75	3.95	201.66	1.30	polar

4-nonylphenol



- moderately soluble in water
- moderately bioaccumulative, not readily biodegradable
- can cause endocrine disruption in fish by interacting with estrogen receptors and androgen receptors, decreases male fertility

Recommendations

- Improved efficiency for sample prep using auto SPE
- Oasis HLB good for polar analytes
- Flow rate of auto SPE too high for 4-nonylphenol
- Load and wash losses
- Subjected to traditional trial-and-error optimization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