

# **SPE Basics: Sorbents, Sorption, and Multiclass Analytes**

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# Arsenal of Sample Preparation Acronyms

- Solid-phase extraction (SPE)
- Liquid-liquid extraction (LLE)
- Liquid-solid extraction (LSE)
- Supercritical fluid extraction (SFE)
- Solid-phase microextraction (SPME)
- Quick, easy, cheap, effective, rugged and safe (QuEChERS)
- Stir-Bar sorptive extraction (SBSE)
- ..... etc.

# **SPE Basics: Sorbents, Sorption, and Multiclass Analytes**

- My SPE Journey
- SPE 101
  - Sorption
  - Hydrophobicity/pH-dependent hydrophobicity
  - Optimization: Recovery too low, Recovery too high,
  - Trying to be all things to all analytes at all concentrations
- Comments on analyses in general: It might not be the SPE!

Google “Principles of Extraction” or  
“Principles of Extraction and the Extraction of  
Semivolatile Organics from Liquids”

- <https://pdfs.semanticscholar.org/22dd/f37cf62989a4a6df9e9388c7d264cbfa2c80.pdf>
- [https://www.researchgate.net/profile/Martha\\_Wells/publication/265925490\\_HANDLING\\_LARGE\\_VOLUME\\_SAMPLES\\_APPLICATIONS\\_OF\\_SPE\\_TO\\_ENVIRONMENTAL\\_MATRICES/links/5576e3cd08ae7521586df82c/HANDLING-LARGE-VOLUME-SAMPLES-APPLICATIONS-OF-SPE-TO-ENVIRONMENTAL-MATRICES.pdf](https://www.researchgate.net/profile/Martha_Wells/publication/265925490_HANDLING_LARGE_VOLUME_SAMPLES_APPLICATIONS_OF_SPE_TO_ENVIRONMENTAL_MATRICES/links/5576e3cd08ae7521586df82c/HANDLING-LARGE-VOLUME-SAMPLES-APPLICATIONS-OF-SPE-TO-ENVIRONMENTAL-MATRICES.pdf)

# Sorption

- **Absorption**
- **Adsorption** (van der Waals/dipole—dipole)
  - Film diffusion (through boundary film surrounding particle)
  - Pore diffusion (within the pore fluid of the particle)
- **Ion-exchange** (electrostatic attraction)
- **Chemical reaction** (covalently bonded) – **Not good for SPE!**
  - Adsorptive reaction (binds, associates, interacts)

## Origins of Intermolecular Attractions

- **Nonspecific (van der Waals interactions)**
  - London dispersive energies (time-varying, uneven electron distributions, i.e., polarizability)
  - Debye energies (dipole—induced dipole interactions)
  - Keesom energies (dipole—dipole interactions)
- **Specific interactions**
  - Electron donor—acceptor interactions
    - Hydrogen bonding

R.P. Schwarzenbach, P.M. Gschwend, and D.M. Imboden, *Environmental Organic Chemistry, Second Edition*, Wiley, New York, 2003, pp. 60—63.

## Mechanisms of retention on activated carbon— a super duper mixed mode sorbent

### Chemical

Van der Waals

Dipole-dipole

Electrostatic

### Physical

Size exclusion

Abouleish, M.Y.Z. and M.J.M. Wells\*. 2015. Trihalomethane Formation Potential of Aquatic and Terrestrial Fulvic and Humic Acids: Sorption on Activated Carbon. *Science of the Total Environment*, 521-522:293-304.

Interaction Mechanism	Interaction Energy
van der Waals	increases ↓
Polar/dipole—dipole	
Hydrogen bonding	
Electrostatic (cation/anion exchange)	

# Solid-phase Extraction: A Disruptive Transformative Revolution

**A multi-staged revolution—in science, technology, cost, time, and safety**

**(1) Developments in the science of sorbents for aqueous samples**

- **Materials Science: Bonded-phase silica materials and others (starting in 1968/1969—to date)**
- **A revolution in the Nernst Distribution Law**

**+**

**(2) Development in technology (devices, columns, disks) for sample preparation (starting in 1978—to date)**

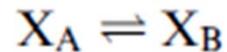
- **A revolution in cost, time, and safety**

# Solid-phase Extraction: A Multi-staged Transformative Revolution

A revolution in an equation.

*Nernst distribution law*

W. Nernst (1891)



$$K_D = \frac{[X]_B}{[X]_A}$$

Chemical component X  
Phase A  
Phase B

If  $K_D$  is large—high degree of extraction from phase A into phase B.

**Optimize extraction** so that the distribution of solute between phases lies far to the right

If  $K_D$  is small—less chemical X is transferred from phase A into phase B

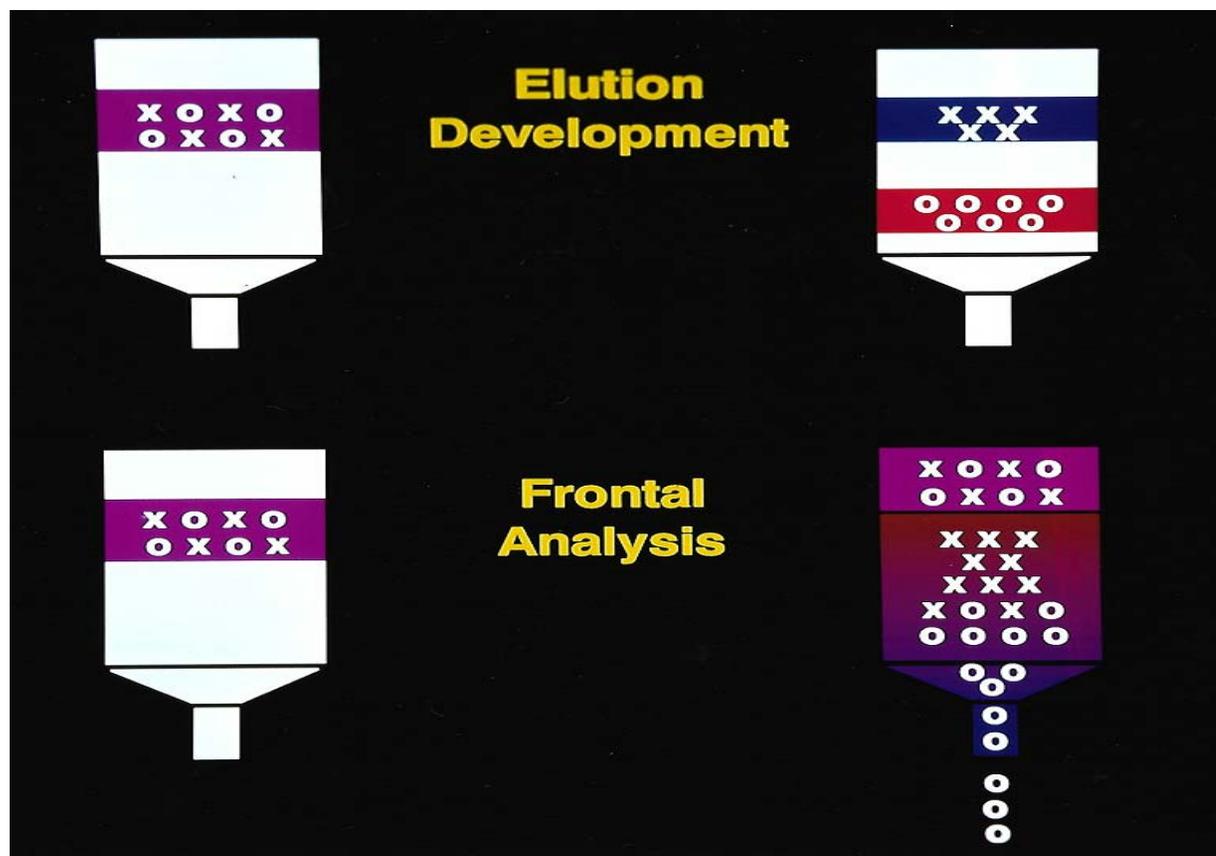
SPE Synonyms	Attribution
All-or-Nothing	Tiselius (1955)/Morris and Morris (1976)
Extraction Chromatography	Braun and Ghersini (1975)
Adsorption Trapping	Ogan et al. (1978)
Solid-Phase Extraction	Zief et al. (1982)/Wachob 1983 (J.T. Baker Chemical Company)
Liquid/Solid, Retention/Elution, Adsorption/Desorption, Stop/Go or Digital Chromatography	Yago (1984)
On/Off Chromatography	Wankat (1986)

$$K_D = \frac{[X]_B}{[X]_A}$$

Many of the terms refer to the nature of SPE in which liquid chromatographic mechanisms are “taken to their extreme” i.e.,  $K_D = 0$  or infinity (Baker 1984)

Simpson, N.\* and M.J.M. Wells. 2000. Introduction to Solid-phase Extraction. In: *Solid-phase Extraction: Principles, Techniques, and Applications*. N. Simpson, Ed., Marcel Dekker, Inc., New York, NY, pp. 1-17.

## SPE is chromatography



90 g bulk C<sub>18</sub> Frontal Analysis

Wells, M.J.M.\* 2000. Essential Guides to Method Development in Solid-phase Extraction. In: *Encyclopedia of Separation Science*. I.D. Wilson, E.R. Adlard, M. Cooke, and C.F. Poole, Eds., Volume 10:4636-4643. Academic Press Ltd, London, UK.

Toxicity Reduction Evaluation (TRE)

Wells, M.J.M. Principles of Extraction and the Extraction of Semivolatile Organics from Liquids. In: *Sample Preparation Techniques in Analytical Chemistry*, S. Mitra, ed., John Wiley & Sons, 2003, pp. 37-138

## Martha's definition:

Solid-phase extraction refers to the **nonequilibrium, exhaustive** removal of chemical constituents from a flowing liquid sample via retention on a contained solid sorbent and subsequent **recovery** of selected constituents by elution from the sorbent.

SPE	Nonequilibrium	Exhaustive
LLE	Equilibrium	Exhaustive
SBSE	Equilibrium	Nonexhaustive
SPME	Equilibrium	Nonexhaustive

## Extraction Method Fundamentals

SPE	Nonequilibrium	Exhaustive
LLE	Equilibrium	Exhaustive
SBSE	Equilibrium	Nonexhaustive
SPME	Equilibrium	Nonexhaustive

**Exhaustive:** Objective is to recover all of the contaminant in the sample

**Nonexhaustive:** Objective is to recover a portion of the contaminant from a sample

## SPE Objectives

1. Concentration
2. Clean-Up
3. Sample Matrix Removal
4. Solvent Exchange

Simpson, N.\* and M.J.M. Wells. 2000. Introduction to Solid-phase Extraction. In: *Solid-phase Extraction: Principles, Techniques, and Applications*. N. Simpson, Ed., Marcel Dekker, Inc., New York, NY, pp. 1-17.

## SPE consists of a basic four-step approach:

1. Sorbent preparation or pre-wash: stationary phase conditioning
2. Retention: analyte adsorption
3. Sorbent post-wash: removing undesirable contaminants
4. Elution: analyte desorption

Wells, M.J.M.\* 2000. Essential Guides to Method Development in Solid-phase Extraction. In: *Encyclopedia of Separation Science*. I.D. Wilson, E.R. Adlard, M. Cooke, and C.F. Poole, Eds., Volume 10:4636-4643. Academic Press Ltd, London, UK.

**SPE**

**Nonequilibrium**

**Exhaustive**

### How to optimize recovery (showing some non-optimized data)

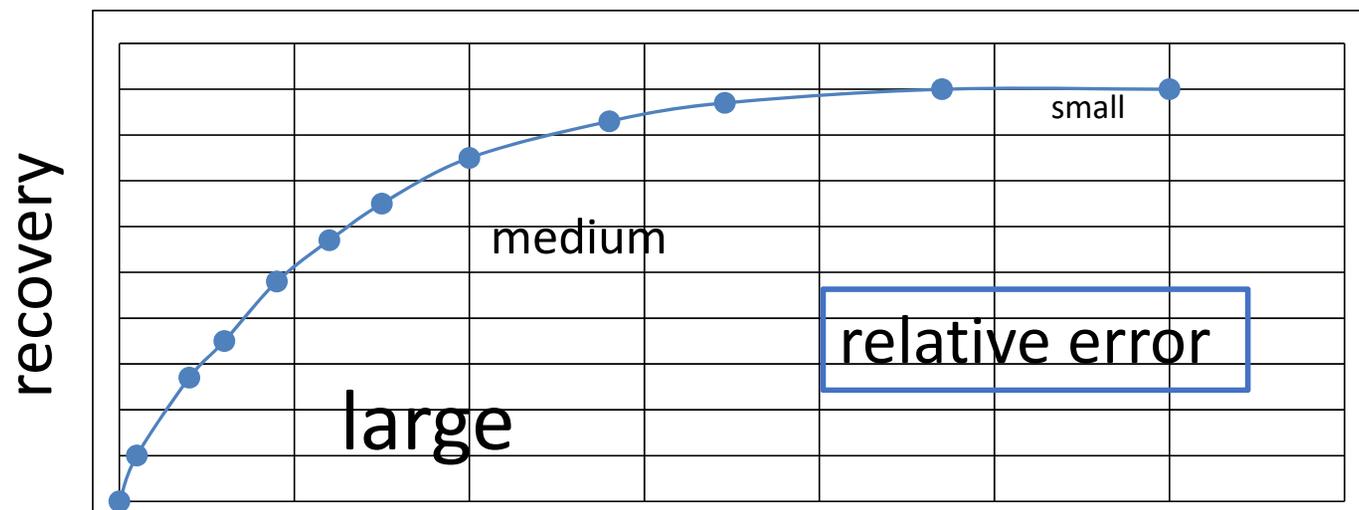
- Know all you can about your analyte(s), the sorbent, and the **matrix**
- “One parameter at a time” approach
  - Control retention/optimize elution
  - Control elution/optimize retention

**recovery = sorption efficiency x desorption efficiency**

If sorption is 50% efficient but desorption is 100% efficient or vice versa, the recovery measured is 50% and it is impossible to know whether sorption or desorption was inefficient or if reduced recovery was produced by a combination of both.

- **Factorial design of multiple parameters**

# Slippery slope



## Equilibrium methods

Time

Temperature

## SPE Non-equilibrium methods

*Retention*

Sorbent type/tactic

Analyte character

Matrix additives

Sample volume

Sorbent mass

*Elution*

Eluting solvent (solvophobic) strength

Eluting solvent volume

Elution rate

# Why you must optimize the SPE method

# SPE Optimization

## A Balancing Act

At a minimum, these are the main parameters to consider

### Retention

Sorbent type/tactic/strategy/maneuver

Analyte character (polarity, hydrophobicity, ionogenicity)

Matrix additives (ion suppression, ion pairing, ionic strength organic solvents)

Sample volume

Sorbent mass

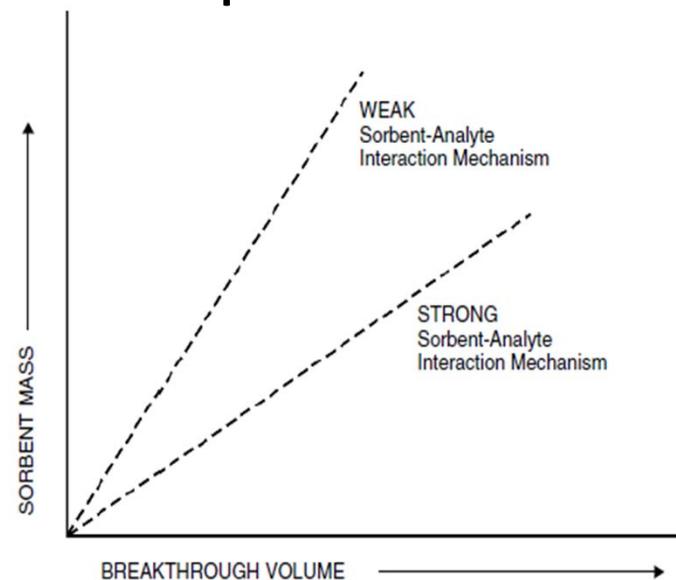
### Elution

Eluting solvent (solvophobic) strength

Eluting solvent volume

Elution rate

### Sample-to-Sorbent Ratio



Wells, M.J.M. Principles of Extraction and the Extraction of Semivolatile Organics from Liquids. In: *Sample Preparation Techniques in Analytical Chemistry*, S. Mitra, ed., John Wiley & Sons, 2003, pp. 37-138

## **Sorbent type/tactic/strategy/maneuver**

**Single-mode sorbents—Selective adsorption/desorption—Reverse elution**

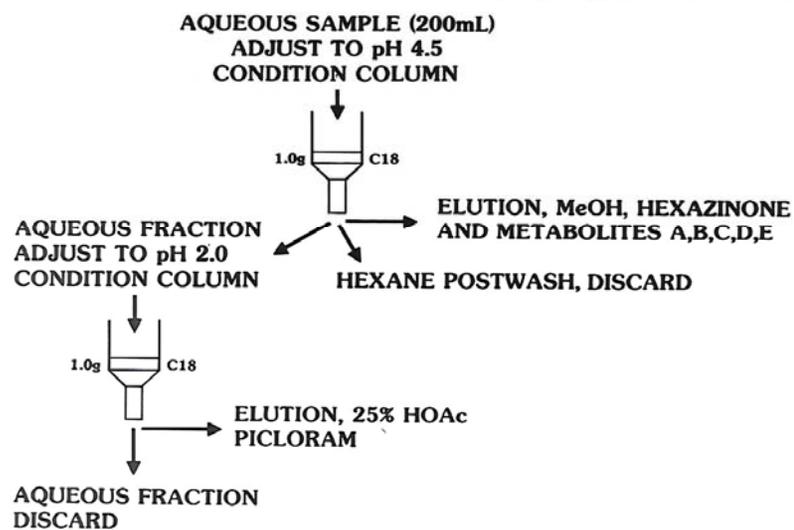
**Multiple-mode sorbents**

**Chromatographic mode sequencing**

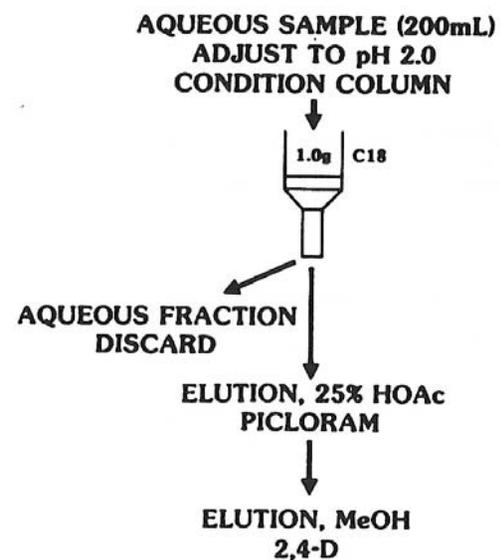
**Mixed-mode sorbents**

# Single-mode sorbents—Selective adsorption/desorption

## EXTRACTION SCHEME HEXAZINONE/HEXAZINONE METABOLITES/PICLORAM



## EXTRACTION SCHEME, PICLORAM/2,4-D

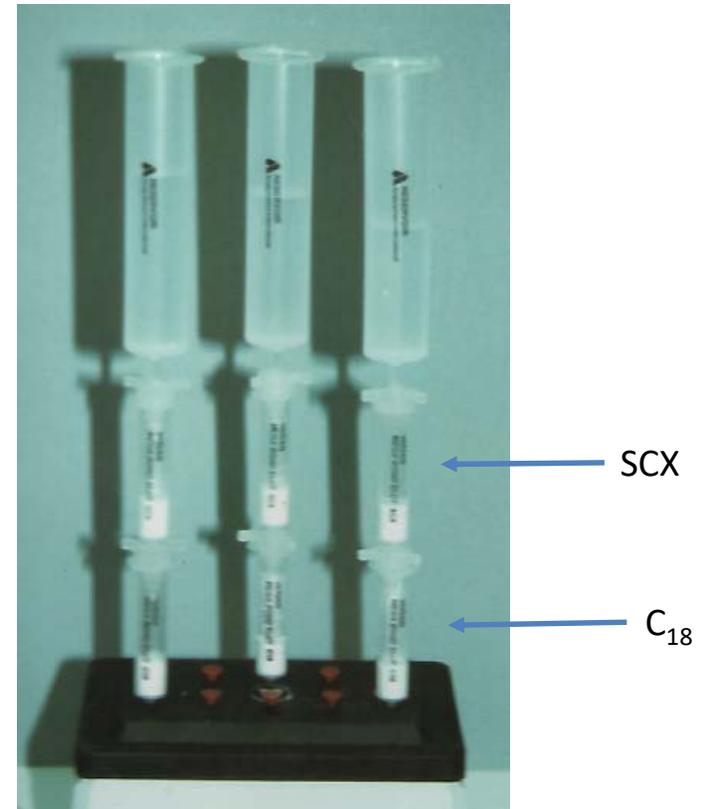
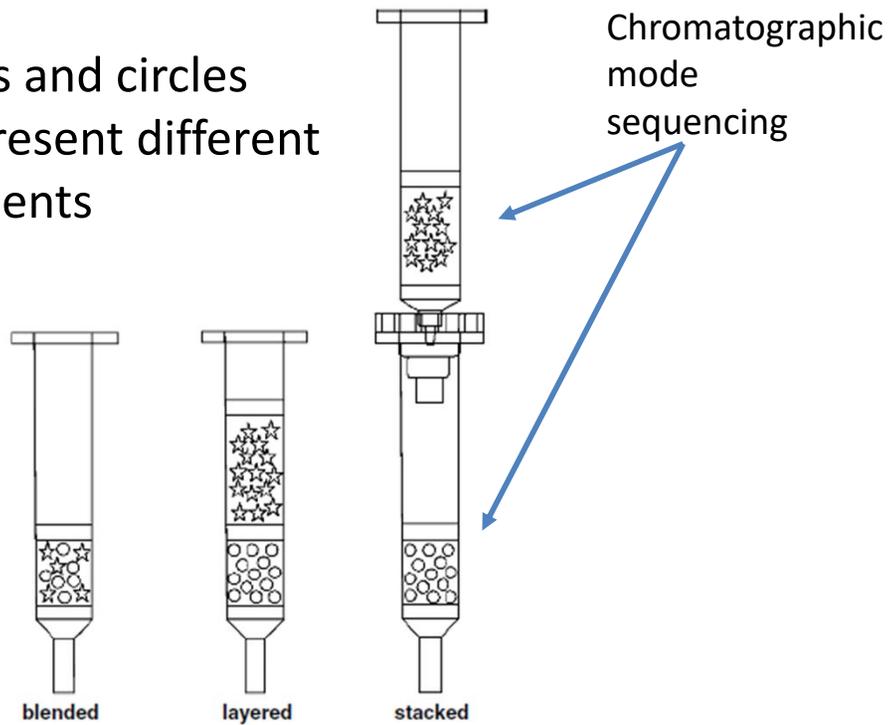


Wells, M.J.M.\* 1986. Off-Line Multistage Extraction Chromatography for Ultrasensitive Herbicide Residue Isolation. In: *Proceedings of the Third Annual International Symposium on Sample Preparation and Isolation Using Bonded Silicas*. Analytichem International, Harbor City, CA, pp. 117-135.

Wells, M.J.M.\* and J.L. Michael. 1987. Recovery of Picloram and 2,4-Dichlorophenoxyacetic Acid from Aqueous Samples by Reversed-Phase Solid-Phase Extraction. *Anal. Chem.* 59:1739-1742.

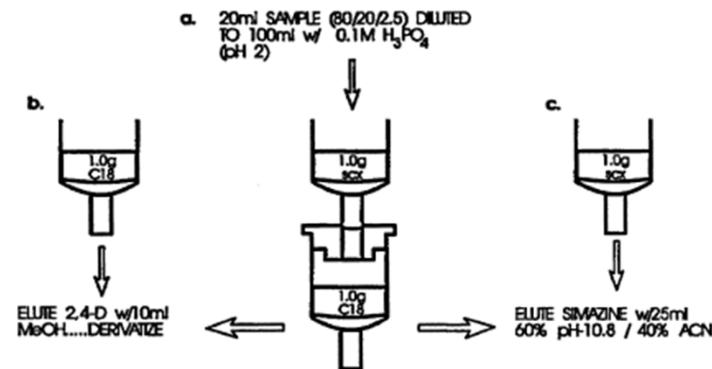
## Multiple-mode sorbents

Stars and circles  
Represent different  
sorbents



## Chromatographic mode sequencing (Selective adsorption)

The objective of the two-column sequence was to adsorb simazine on the strong cation exchange (SCX) column and 2,4-D on the C18 column, thereby separating the compounds.



Sutherland, D.J., G.K. Stearman, and M.J.M. Wells\*. 2003. Development of an Analytical Scheme for Simazine and 2,4-D in Soil and Water Runoff from Ornamental Plant Nursery Plots. *J. Agric. Food Chem.*, 51:14-20.

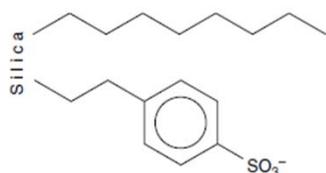
## Reverse elution

Another sorbent manipulation used was reverse elution ensued (in which the direction of elution from the sorbent was reversed to the direction of sample sorption on the sorbent).

J.L. Norman and M.J.M. Wells, unpublished results

## Mixed-mode sorbents

Different functionalities on the same backbone sorbent particle



**Mechanisms of retention on activated carbon—a super duper mixed mode sorbent**

### Chemical

Van der Waals

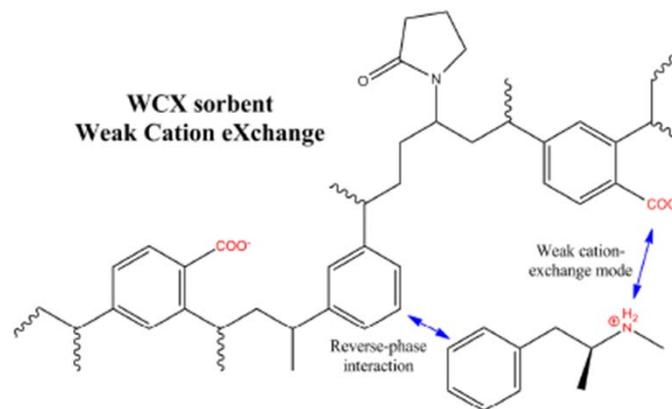
Dipole-dipole

Electrostatic

### Physical

Size exclusion

Abouleish, M.Y.Z. and M.J.M. Wells\*. 2015. Trihalomethane Formation Potential of Aquatic and Terrestrial Fulvic and Humic Acids: Sorption on Activated Carbon. *Science of the Total Environment*, 521-522:293-304.



### WCX – methamphetamine interaction

(WCX structure adapted from

<http://www.waters.com/webassets/cms/library/docs/720001692en.pdf>)

Boles, T.H. and M.J.M. Wells\*. 2016. Analysis of Amphetamine and Methamphetamine in Municipal Wastewater Influent and Effluent using Weak Cation-exchange Solid-phase Extraction (SPE) and Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). *Electrophoresis*, 37(23–24):3101–3108.

# SPE Optimization

At a minimum, these are the main parameters to consider

## *Retention*

Sorbent type/tactic/strategy/maneuver

Analyte character (polarity, hydrophobicity, ionogenicity)

Matrix additives (ion suppression, ion pairing, ionic strength organic solvents)

Sample volume

Sorbent mass

## *Elution*

Eluting solvent (solvophobic) strength

Eluting solvent volume

Elution rate

**Of all the relevant solute properties, five chemical properties are fundamental to understanding extraction theory:**

- Vapor pressure
  - Solubility
  - Molecular weight
  - Hydrophobicity ( $\log P$ ,  $\log K_{ow}$ )
  - Acid dissociation ( $pK_a$ )
- } pH-dependent hydrophobicity ( $\log D$ )

**These essential properties determine the transport of chemicals:**

- in the human body (Medicinal Chemistry)
- in the air/water/soil environmental compartments (Environmental Chemistry)
- between immiscible phases during analytical extraction (Analytical Chemistry)

Wells, M.J.M. Principles of Extraction and the Extraction of Semivolatile Organics from Liquids. In: *Sample Preparation Techniques in Analytical Chemistry*, S. Mitra, ed., John Wiley & Sons, 2003, pp. 37-138

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**Vapor pressure:** Careful drying sample extracts

Many analytes are non-volatile or semi-volatile, but some are more volatile than most.

Hydrophobic, yet volatile, character

Trifluralin

\*Vapor Pressure 1.21E-5 Torr at 25 degrees C

\*Log P = 4.558

Trifluralin has a high vapor pressure (U.S. EPA, 1989), so it is volatile enough for direct GC analysis without derivatization.

\*Calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2018 ACD/Labs)

Garimella, U.I., G.K. Stearman, and M.J.M. Wells\*. 2000. Comparison among Soil Series and Extraction Methods for the Analysis of Trifluralin. *J. Agric. Food Chem.*, 48:5874-588.

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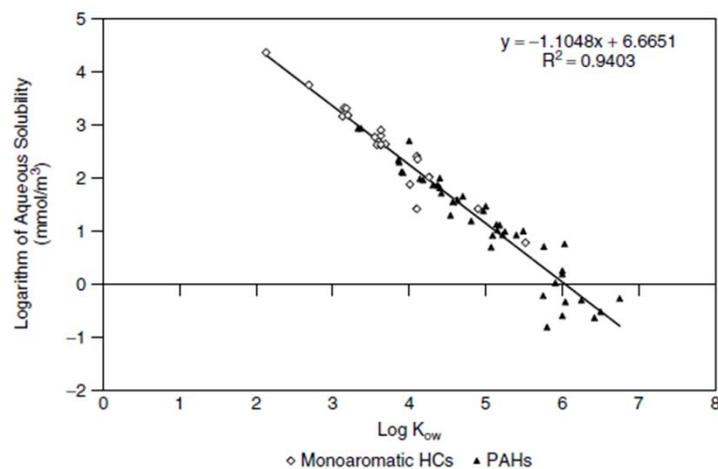
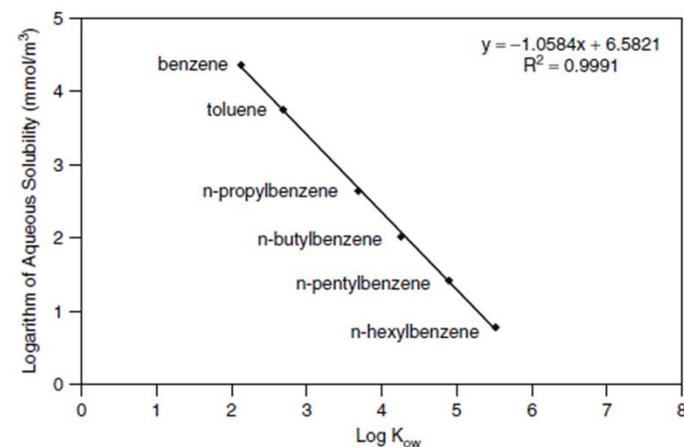
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# Water solubility and hydrophobicity

- There is a trend toward an inverse relationship between these parameters such that high water solubility is generally accompanied by low hydrophobicity, and vice versa.
- Many authors use this relationship to estimate one of these parameters from the other.
- However, the n-octanol/water partition coefficient and water solubility are not interchangeable (via inverse relationships) because **they measure different phenomena**.
- Water solubility is a property measured at maximum capacity or saturation.
- The n-octanol/water partition coefficient measures distribution across an interface.
- While the relationship between water solubility and the n-octanol/water partition coefficient may be highly correlated for closely related families of congeners, as the diversity of the compounds compared increases, the correlation between these two parameters decreases.



Wells, M.J.M. Principles of Extraction and the Extraction of Semivolatile Organics from Liquids. In: *Sample Preparation Techniques in Analytical Chemistry*, S. Mitra, ed., John Wiley & Sons, 2003, pp. 37-138

Of all the relevant solute properties, five chemical properties are fundamental to understanding extraction theory:

- Vapor pressure
  - Solubility
  - **Molecular weight**
  - Hydrophobicity ( $\log P$ ,  $\log K_{ow}$ )
  - Acid dissociation ( $pK_a$ )
- Henry's Law constant; molar concentration
- pH-dependent hydrophobicity ( $\log D$ )

These essential properties determine the transport of chemicals:

- in the human body (Medicinal Chemistry)
- in the air/water/soil environmental compartments (Environmental Chemistry)
- between immiscible phases during analytical extraction (Analytical Chemistry)

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The theory of **chemical equilibrium** leads us to describe the reversible distribution reaction as



and the equilibrium constant expression is

$$K_D = \frac{[X]_B}{[X]_A}$$

The general equilibrium constant expression can be rewritten to express the distribution of chemical X between water (W) and *n*-octanol (O) as

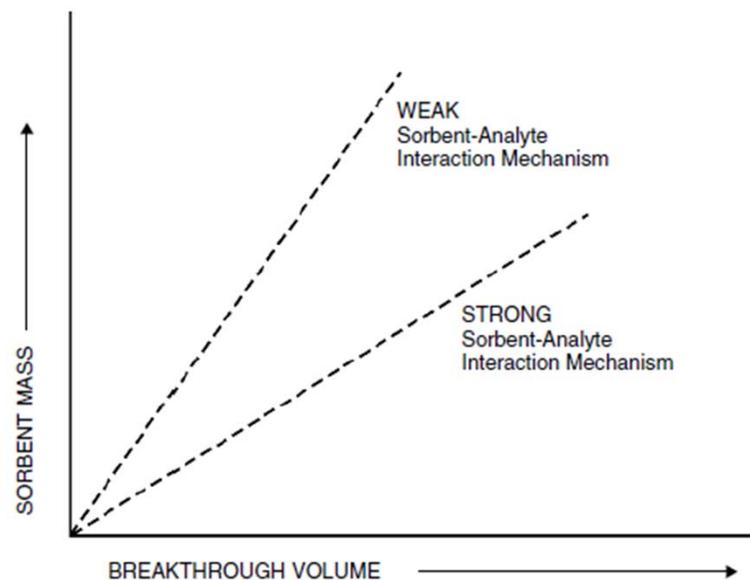
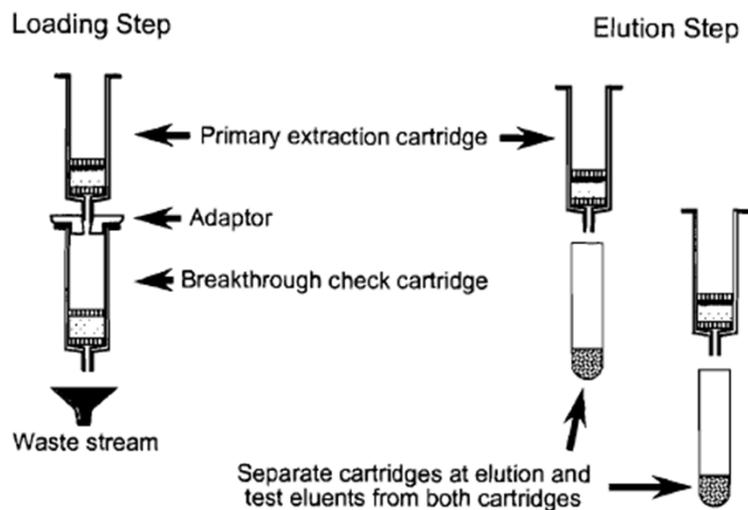
$$K_{OW} = K_D = \frac{[X]_O}{[X]_W} \quad \begin{array}{l} \text{P, Partition Coefficient} \\ \text{Log P} \end{array}$$

**But be careful!!! Most current data are computer-generated and refer to the nonionized form of the compound.**

## Hydrophobicity/hydrophilicity Lipophilicity/lipophobicity

The *n*-octanol/water partition coefficient,  $K_{OW}$  (also referred to as  $P_{ow}$ ,  $P$ , or  $P_{oct}$ , is a dimensionless operational definition of hydrophobicity based on the *n*-octanol reference system. The differences in  $K_{ow}$  cover several orders of magnitude such that hydrophobicity values are often reported on a logarithmic scale.

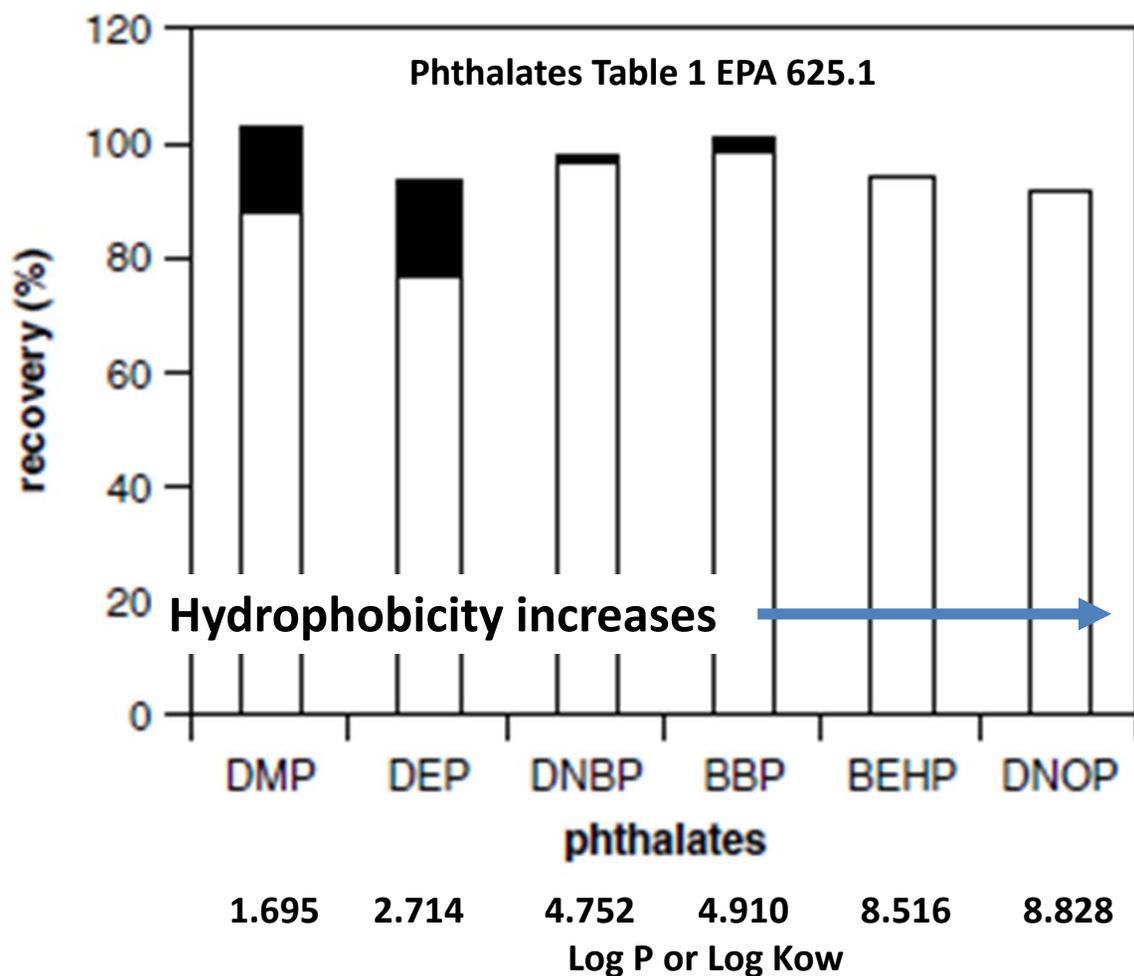
For nonionic or nonionizable compounds uncharged at all pHs,  $K_{ow}$  or  $P$  is not pH-dependent, i.e., the same at all pHs.



**Using a second cartridge in series with the primary extraction cartridge provides a simple indicator of breakthrough.**

Wells, M.J.M.\* 2000. Handling Large Volume Samples: Applications of SPE to Environmental Matrices. In: *Solid-phase Extraction: Principles, Techniques, and Applications*. N. Simpson, Ed., Marcel Dekker, Inc., New York, NY, 97-123.

**recovery = sorption efficiency x desorption efficiency**



Very hydrophilic  
Very hydrophobic

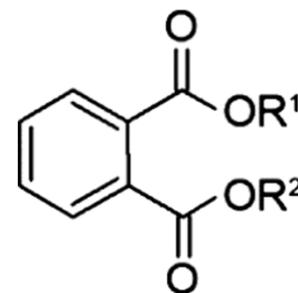
Recovery too low

■ lower column  
□ upper column



Sorbent mass  
Sorbent: 2.0 g C<sub>8</sub>

Polar  
Non-ionizable  
Log P or Log Kow = Log D  
pH-independent hydrophobicity



Phthalic diester

Calculated using Advanced Chemistry Development (ACD/Labs)  
Software V11.02 (© 1994-2018 ACD/Labs)

## Water solvates slower than expected

“Given how common water is, you’d think by now we would know just about everything there is to know about it. Not so.”

“A new study shows that when it comes to orienting around a solute, water molecules are a lot more sluggish than generally thought.”

Mitch Jacoby, C&EN 96(28) 6. (July 9, 2018)

J Chem. Phys. 2018, DOI:10.1063/1.5034225

- **Film diffusion (through boundary film surrounding particle)**

For certain compounds (phthalates, chlorobenzenes, oil and grease, herbicides), as the hydrophobicity of the solute increased, so did the importance of allowing elution to occur by gravity rather than by the normal mode of vacuum elution. The effect appears to result from slow mass transfer for very hydrophobic compounds from the stationary phase into the mobile phase.

2<sup>3</sup> FACTORIAL DESIGN MATRIX

Run type	pH	Eluotropic strength	Ionic strength	Added MeOH	Vacuum/gravity
1	2	MeOH	–	–	Vacuum
2	7	MeOH	–	–	Vacuum
3	2	EtOAc	–	–	Vacuum
4	7	EtOAc	–	–	Vacuum
5	2	MeOH	NaCl	–	Vacuum
6	7	MeOH	NaCl	–	Vacuum
7	2	EtOAc	NaCl	–	Vacuum
8	7	EtOAc	NaCl	–	Vacuum
9	2	MeOH	–	20	Vacuum
10	7	MeOH	–	20	Vacuum
11	2	EtOAc	–	20	Vacuum
12	7	EtOAc	–	20	Vacuum
13	2	MeOH	NaCl	20	Vacuum
14	7	MeOH	NaCl	20	Vacuum
15	2	EtOAc	NaCl	20	Vacuum
16	7	EtOAc	NaCl	20	Vacuum
17	2	MeOH	–	–	Gravity
18	7	MeOH	–	–	Gravity
19	2	EtOAc	–	–	Gravity
20	7	EtOAc	–	–	Gravity
21	2	MeOH	NaCl	–	Gravity
22	7	MeOH	NaCl	–	Gravity
23	2	EtOAc	NaCl	–	Gravity
24	7	EtOAc	NaCl	–	Gravity
25	2	MeOH	–	20	Gravity
26	7	MeOH	–	20	Gravity
27	2	EtOAc	–	20	Gravity
28	7	EtOAc	–	20	Gravity
29	2	MeOH	NaCl	20	Gravity
30	7	MeOH	NaCl	20	Gravity
31	2	EtOAc	NaCl	20	Gravity
32	7	EtOAc	NaCl	20	Gravity

For metribuzin, if elution is done under vacuum, methanol is the preferred elution solvent. If elution is allowed to occur by gravity, ethyl acetate produces better recovery.

Wells, M.J.M.\*, D.D. Riemer, and M.C. Wells-Knecht. 1994. Development and Optimization of a Solid-Phase Extraction Scheme for Determination of the Pesticides Metribuzin, Atrazine, Metolachlor and Esfenvalerate in Agricultural Runoff Water. *J. Chromatogr. A.* 659:337-348.

# Extrathermodynamic

QSARs (quantitative structure-activity relationships)

QSRRs (quantitative structure-retention relationships)

Extended Hansch Equation

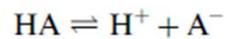
$$\log(1/C) = -k_1(\log P)^2 + k_2 \log P + k_3 \sigma + k_4 E_S + k_5$$

Hansch, Corwin. *Accounts of Chemical Research* (1969), 2(8), 232-9.

As much as hydrophobicity is a yin-yang driving life force, it does not explain everything.

# pH-dependent ionization of analytes

Dissociation of a weak acid, HA



Equilibrium constant for dissociation of a weak acid

$$K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}$$

Take the negative logarithm of both sides to derive the Henderson—Hasselbalch equation

$$\text{pH} = \text{p}K_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

**Don't forget about the pK<sub>a</sub> of the sorbent!!!**

Relationship between system pH and the ratio of ionized to nonionized species

## $D_{ow}$

There is a **pH-dependent distribution** between water and the organic phase in the ionized and nonionized forms

Only the neutral form of the compound partitions:

$$\log D(\text{pH}) = \log P - \log(1 + 10^{(\text{pH} - \text{pK}_a)\Delta_i})$$

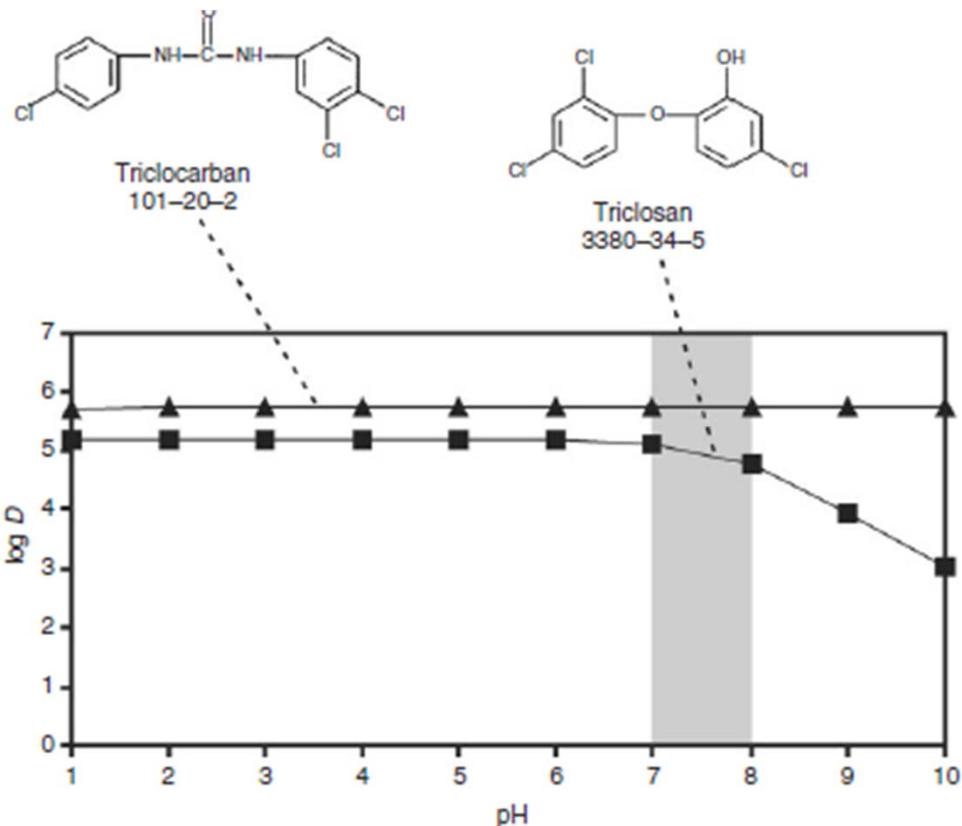
Tetko and Poda, 2004; Hansch and Leo, 1995

Includes ion pair partitioning:

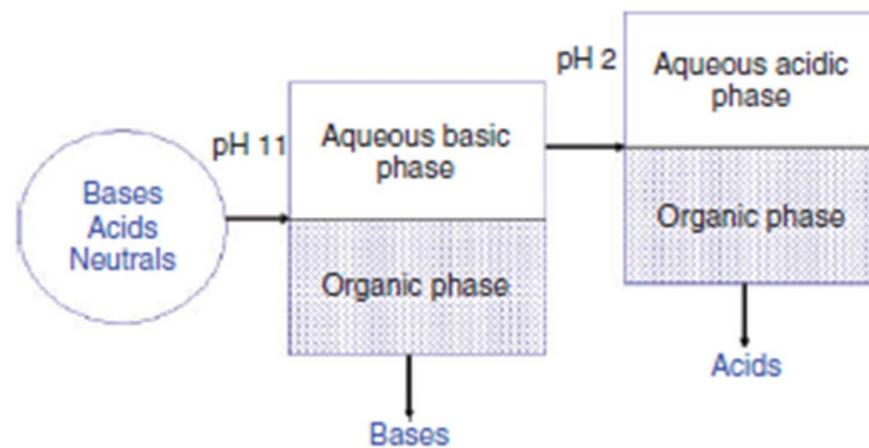
$$D_{p[H]} = \frac{[\text{unionized} + \text{ionized species}]_{\text{octanol}}}{[\text{unionized} + \text{ionized species}]_{\text{water}}}$$

Van de Waterbeemd, Lennernas and Artursson, *Drug Bioavailability*, Wiley-VCH, 2003.

# Hydrophobicity—ionogenicity profile



## US EPA Method 625 Organic base/neutrals and acids Liquid-liquid extraction (LLE)



$$D_{pH} = \frac{[\text{nonionized} + \text{ionized species}]_{\text{octanol}}}{[\text{nonionized} + \text{ionized species}]_{\text{water}}}$$

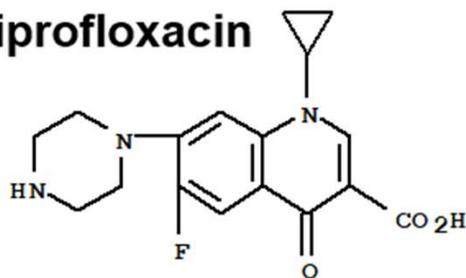
Wells, M.J.M. 2013. Letter to the Editor: Good Riddance, Triclosan. *Chemical and Engineering News*, 91(5), 2.

Wells, M.J.M.\* 2007. Examination of the Mobility Scoring Hierarchy Used to Select Chemicals for the U.S. EPA Contaminant Candidate List Classification Procedure [CD-ROM pp. 86-98]. Proceedings of the Water Environment Federation 2007 Specialty Conference Series. Compounds of Emerging Concern: What Is on the Horizon? Providence, RI.

Calculated versus Measured log P

**Caution!** Calculated log P is for the Neutral Compound!

Ciprofloxacin



log P = 1.31

pK<sub>a</sub> = 6.03, 8.38

Measured log P (Hansch et al., 1995)

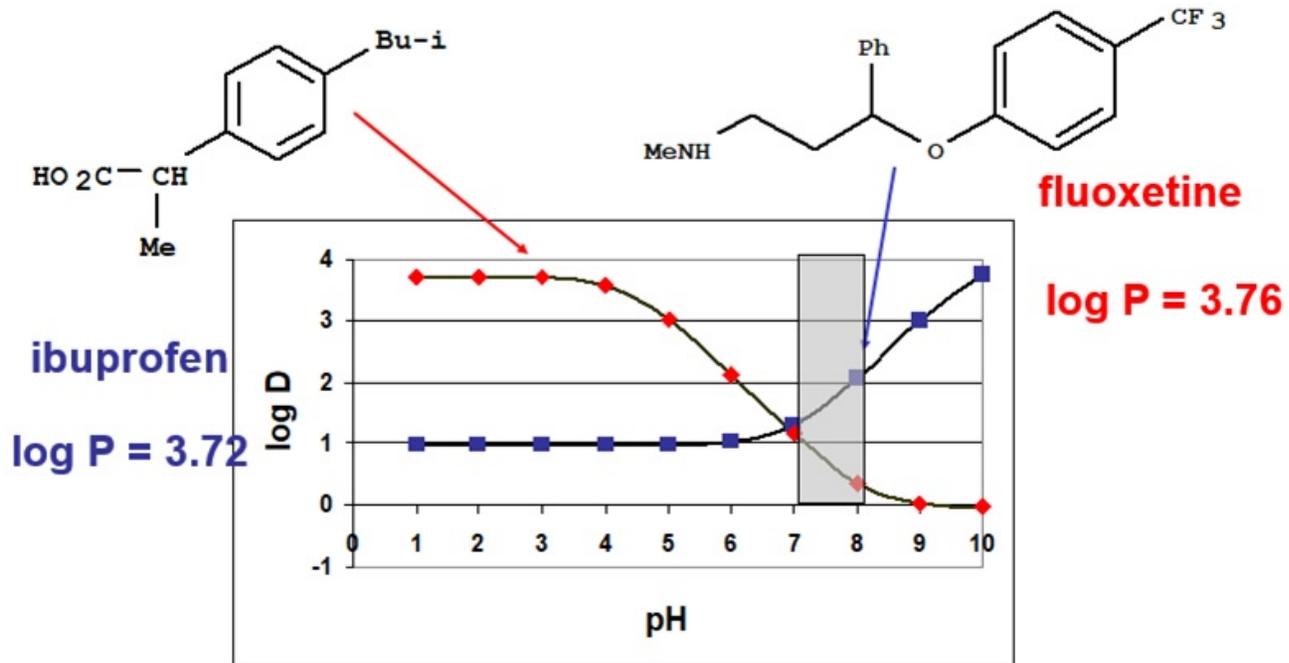
pH 7.2	pH 7.4	pH 7.2
-0.70	-1.11	-1.70

Optimum value = -1.08

pH	log D
1	-1.80
2	-1.79
3	-1.78
4	-1.75
5	-1.54
6	-1.07
7	-0.85
8	-0.95
9	-1.47
10	-2.14

**But be careful!!!** Most current data are computer-generated and refer to the nonionized form of the compound.

Calculated data in this presentation were obtained using SciFinder Scholar Calculated using Advanced Chemistry Development (ACD/Labs) Software Solaris V8.14

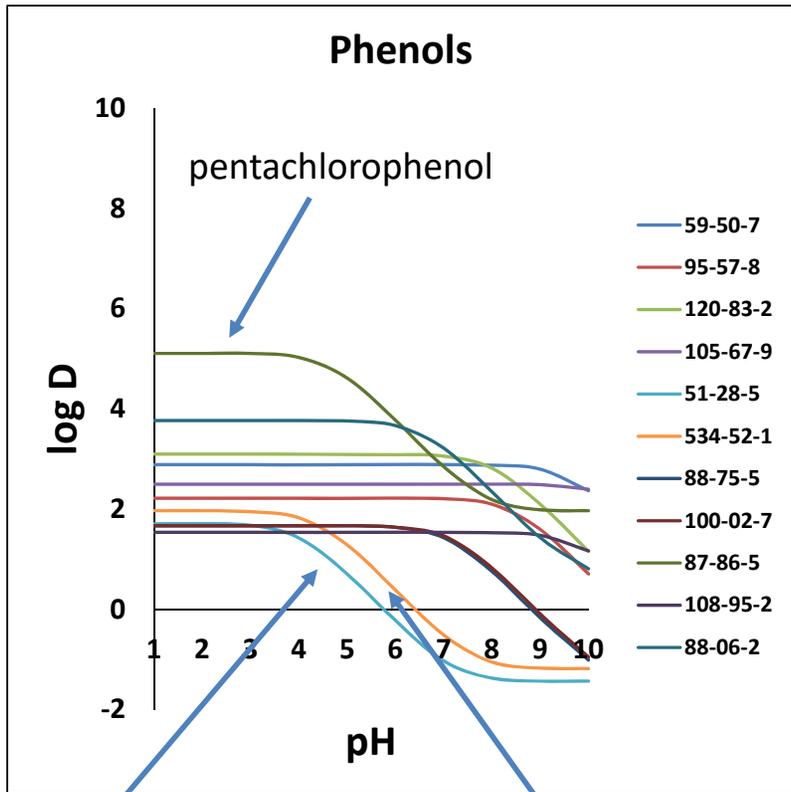


Acids and bases have opposite pH—hydrophobicity profiles

Phthalates Table 1 EPA 625.1

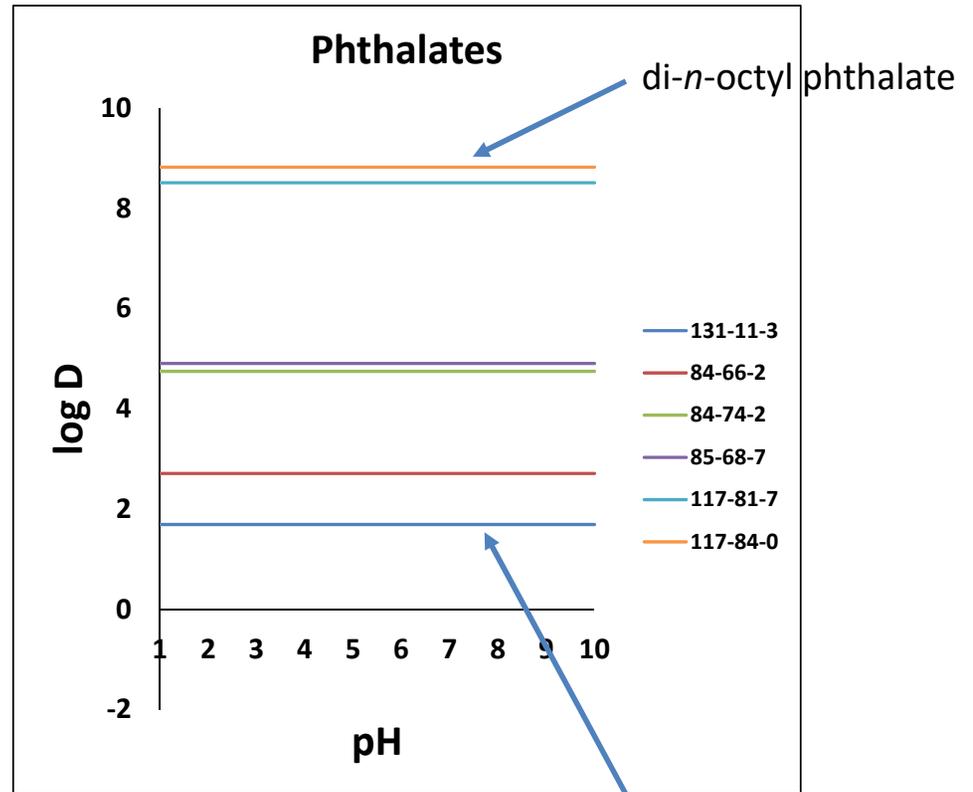
Phenols Table 2 EPA 625.1

pH—log D profiles



2,4-dinitrophenol

2-methyl-4,6-dinitrophenol



dimethyl phthalate

## Sorbents, Sorption, and **Multiclass** Analytes

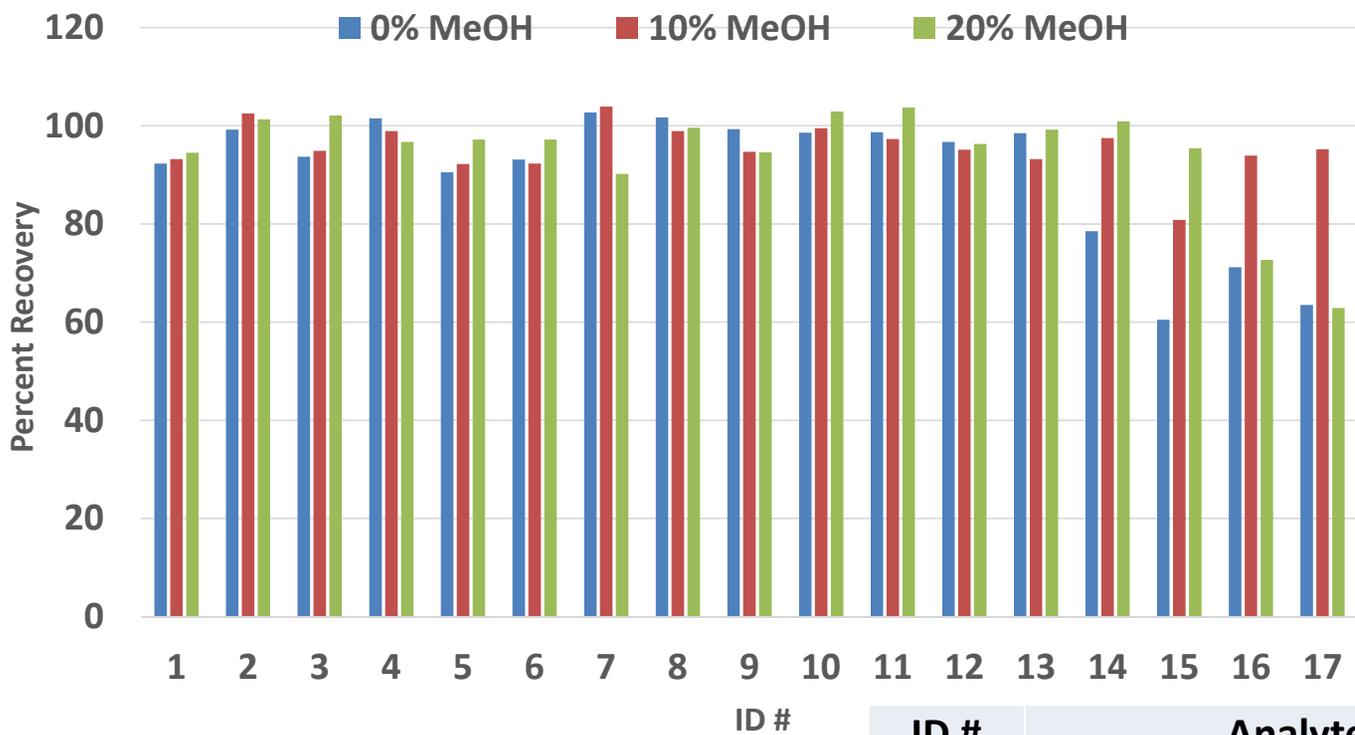
<u>ID #</u>	<u>Analyte</u>	<u>CAS Registry #</u>	<u>log D @ pH2</u>
1	phenol	108-95-2	1.54
2	2-nitrophenol	88-75-5	1.67
3	4-nitrophenol	100-02-7	1.67
4	dimethyl phthalate	131-11-3	1.695
5	2,4-dinitrophenol	51-28-5	1.71
6	2-methyl-4,6-dinitrophenol	534-52-1	1.97
7	2-chlorophenol	95-57-8	2.22
8	2,4-dimethylphenol	105-67-9	2.5
9	diethyl phthalate	84-66-2	2.714
10	4-chloro-3-methylphenol	59-50-7	2.89
11	2,4-dichlorophenol	120-83-2	3.1
12	2,4,6-trichlorophenol	88-06-2	3.77
13	di-n-butylphthalate	84-74-2	4.752
14	benzyl butyl phthalate	85-68-7	4.91
15	pentachlorophenol	87-86-5	5.11
16	bis(2-ethylhexyl) phthalate	117-81-7	8.516
17	di-n-octyl phthalate	117-84-0	8.828

Phthalates Table 1 EPA 625.1

Phenols Table 2 EPA 625.1

Calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2018 ACD/Labs) SciFinder Scholar, 2018 Edition, American Chemical Society

## phenols/phthalates



Sample: 25 mL with/without added Methanol (MeOH)  
Sorbent: 0.6 g Styrene-divinylbenzene copolymer

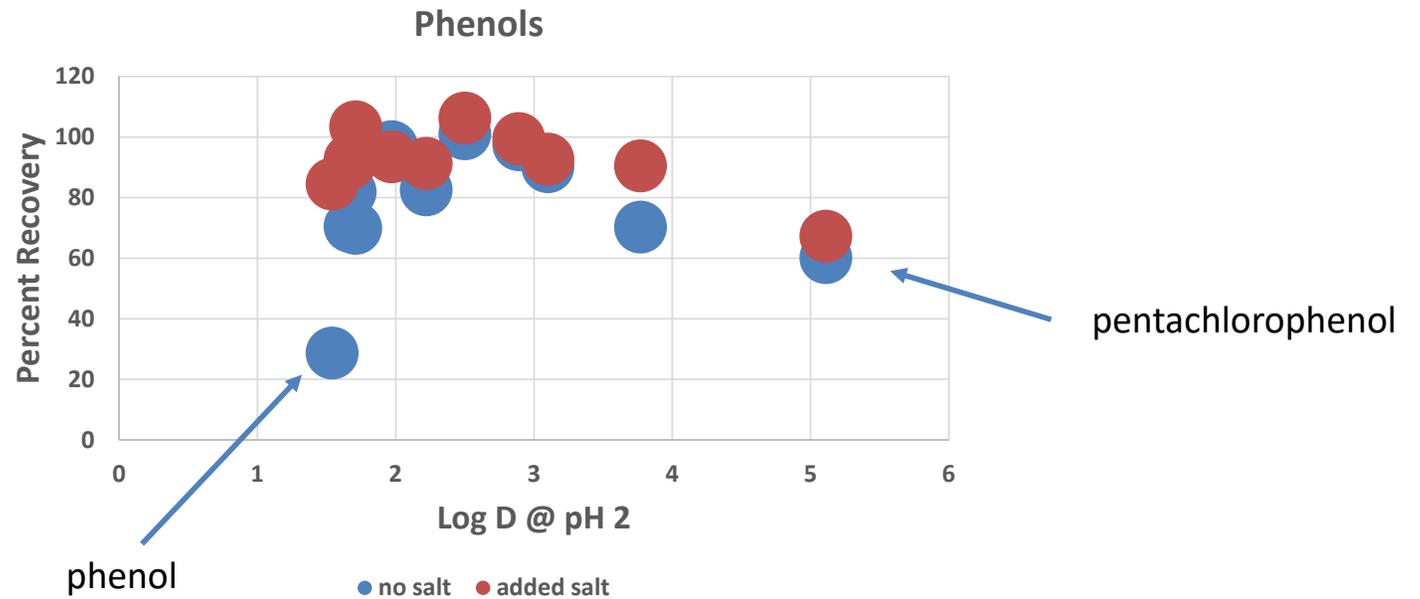
H. Yuan and M. J. M. Wells, unpublished data

Matrix Additive—organic solvent

ID #	Analyte	log D @ pH2
14	benzyl butyl phthalate	4.91
15	pentachlorophenol	5.11
16	bis(2-ethylhexyl) phthalate	8.516
17	di-n-octyl phthalate	8.828

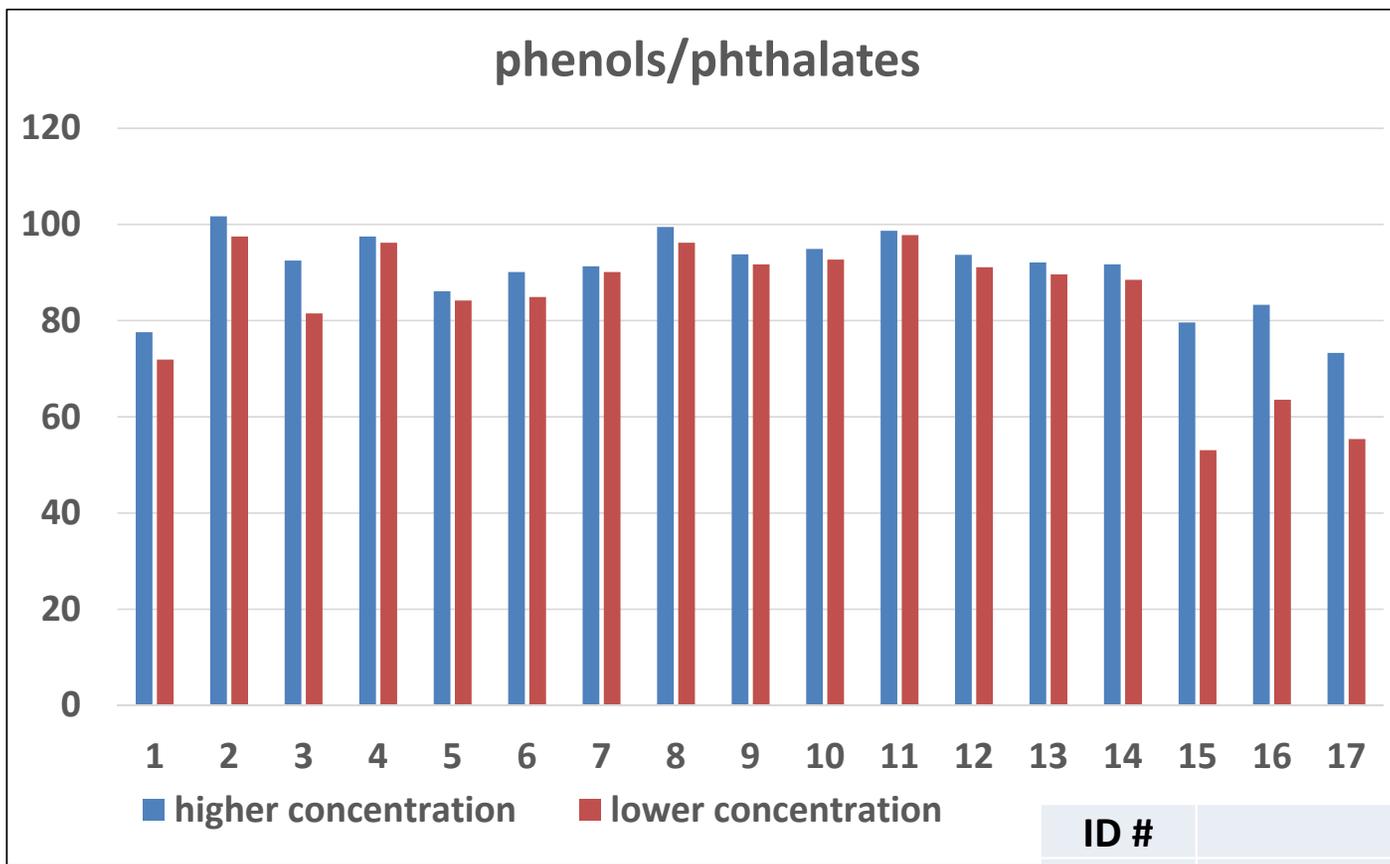
# Hydrophilic—hydrophobic extremes

## Matrix Additive—salt



H. Yuan and M. J. M. Wells, unpublished data

Sample: 50 mL with/without added salt  
Sorbent: 2.0 g Cyclohexyl



Sample: 1 L with added 10% Methanol (MeOH)  
Sorbent: 0.6 g Styrene-divinylbenzene copolymer

H. Yuan and M. J. M. Wells, unpublished data

**Concentration dependence is the kiss of death!!!**

ID #	Analyte	log D @ pH2
14	benzyl butyl phthalate	4.91
15	pentachlorophenol	5.11
16	bis(2-ethylhexyl) phthalate	8.516
17	di-n-octyl phthalate	8.828

# Optimization summary

## *Retention*

Sorbent type/tactic

Analyte character

Matrix additives

Sample volume

Sorbent mass

## *Elution*

Eluting solvent (solvophobic) strength

Eluting solvent volume

Elution rate

- “One parameter at a time” approach
- Factorial design of multiple parameters

As much as hydrophobicity is a yin-yang driving life force, it does not explain everything.

# MISCELLANEOUS!!!

**Recovery too low?**

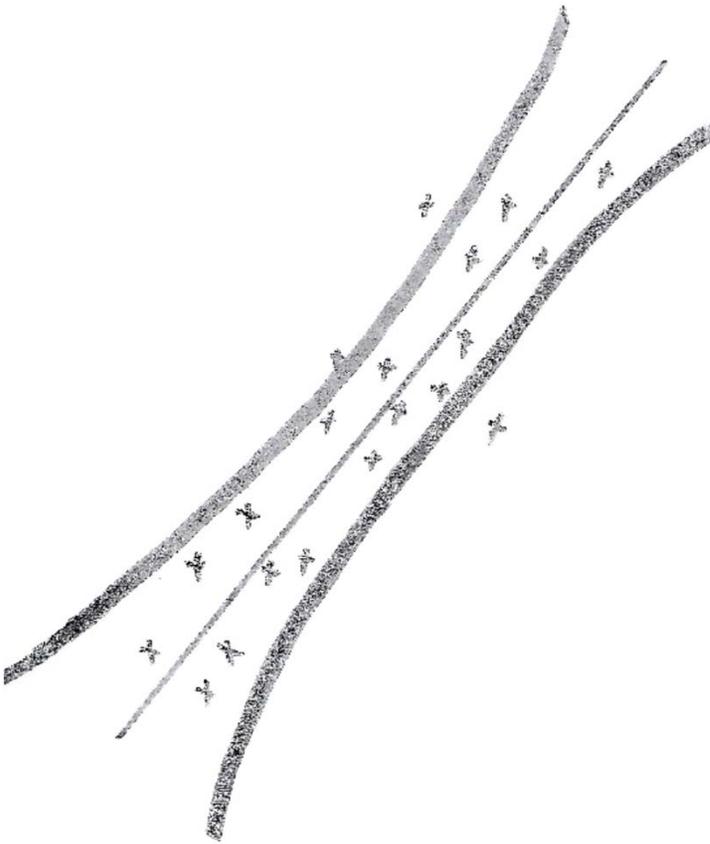
**Recovery too high?**

**Broad acceptance values?**

**It might not be the SPE!**

## Calibration Plot Confidence Intervals

### You can't fight statistics

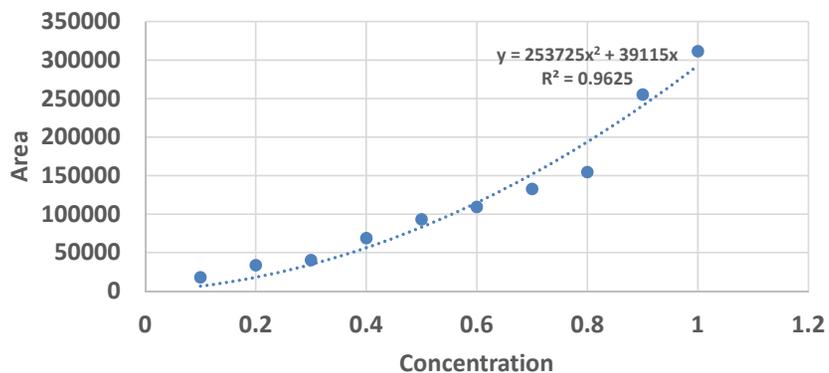


The further the  $x$  value is from the center of the data the more variable is the estimate. The interval width is a minimum for the mean and widens as the distance from the mean increases; therefore, the precision of estimation deteriorates.

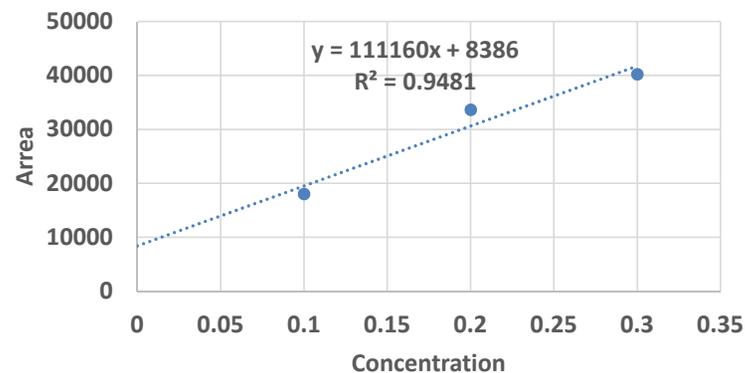
D.C. Montgomery, E.A. Peck, and G.G. Vining. Introduction to Linear Regression Analysis. John Wiley & Sons, Inc. Hoboken, NJ 2012.

**Don't assume the calibration plot is linear!!!  
It might be quadratic (polynomial).**

**Trifluralin GC Calibration Plot**



**Hypothetical Calibration Plot**



**Don't trust everything to the computer.  
Look at the chromatograms.**

U.I. Garimella and M.J.M. Wells, unpublished results

# Stability of Standards and Extracts

## Degradation products of trifluralin and its metabolites

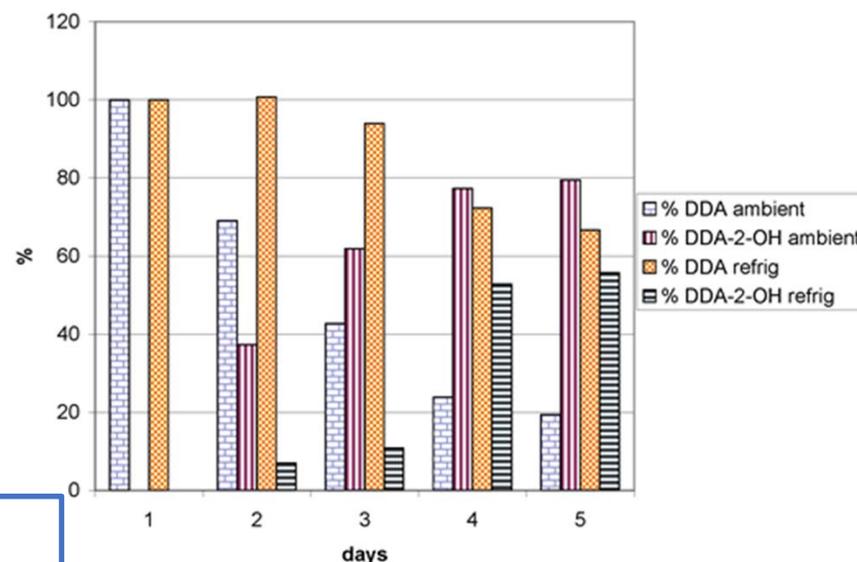
Age (Week)	stds	TR1	TR2	TR3	TR4	TR5	TR6
Zero	TR1	✓					
	TR2		✓				
	TR3			✓			
one	TR1	✓		✓		✓	✓
	TR2		✓			✓	✓
	TR3			✓	✓	✓	✓
two	TR1	✓				✓	✓
	TR2		✓			✓	
	TR3			✓			✓
three	TR1	✓			✓		✓
	TR2		✓			✓	✓
	TR3			✓	✓	✓	

Trifluralin  
Simazine  
Table 3 EPA 625.1

✓ Major compound; ✓ Minor compound

U.I. Garimella and M.J.M. Wells, unpublished results

Acid-catalyzed hydrolytic conversion of DDA to DDA-2-OH at acidic pH.



Metabolites of simazine:  
deethyldeisopropylatrazine (DDA)  
1,3,5-triazin-2-ol, 4,6-diamino (DDA-2-OH)

Standards and reconstituted extracts were not stable in acidic media. Elevating pH levels to circumneutral preserved the samples.

J.L. Norman and M.J.M. Wells, unpublished results

## Stability of Field Samples

Chlorsulfuron and sulfometuron methyl are unstable over time at reduced pH. Aqueous samples had to be stabilized in the field by adding buffer immediately to grab samples or preplaced in field sampler bottles.

Also, chlorsulfuron and sulfometuron methyl are less stable in methanol solution than in acetonitrile.

Wells, M.J.M. and J.L. Michael. 1987. Reversed-Phase Solid-Phase Extraction for Aqueous Environmental Sample Preparation in Herbicide Residue Analysis. *J. Chromatogr. Sci.* 25:345-350.

# Reconstituting and Equilibrating Samples

When samples are evaporated to dryness...

for LC, reconstitute the sample in the initial gradient [analytical mobile phase](#), i.e., not just methanol or acetonitrile and allow the sample to stand overnight (refrigerated)

split peaks question in [Research Gate](#)

Split peaks and poor peak shape have been observed previously in this laboratory for analytes dissolved in pure methanol or acetonitrile injected into aqueous/organic mixtures of HPLC solvents. As the sample plug in pure solvent begins to mix with the aqueous solvent mixture, some of the analyte travels ahead of the bulk sample and some lags behind mixing with the HPLC solvent resulting in the poor performance observed.

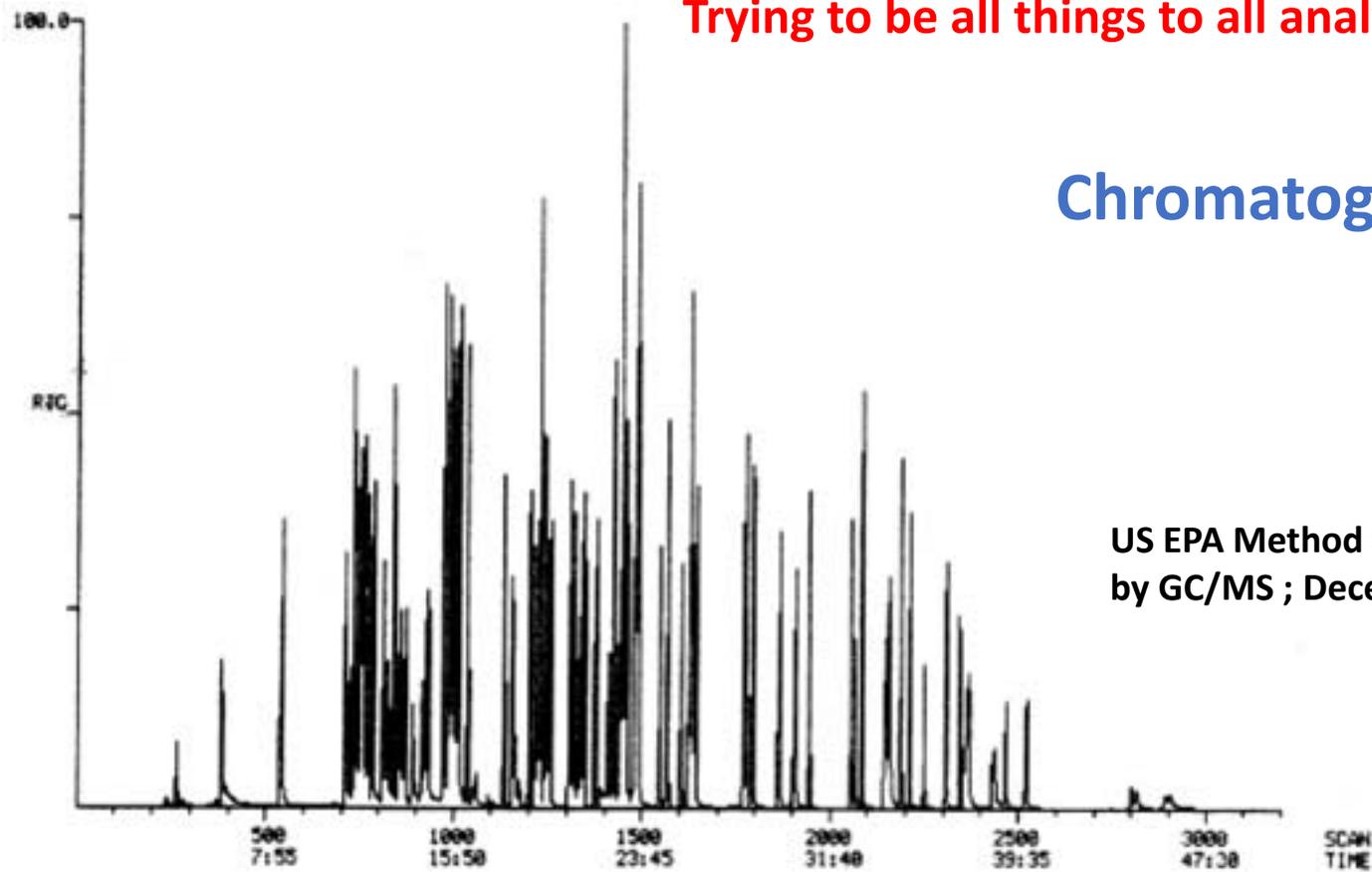
This reconstituted solution is allowed to stand overnight (refrigerated) to allow equilibration before injection or transfer to other vials. Spurious results may be obtained if the samples are transferred immediately after reconstitution.

J.L. Norman and M.J.M. Wells, unpublished results

Sutherland, D.J., G.K. Stearman, and M.J.M. Wells\*. 2003. Development of an Analytical Scheme for Simazine and 2,4-D in Soil and Water Runoff from Ornamental Plant Nursery Plots. *J. Agric. Food Chem.*, 51:14-20.

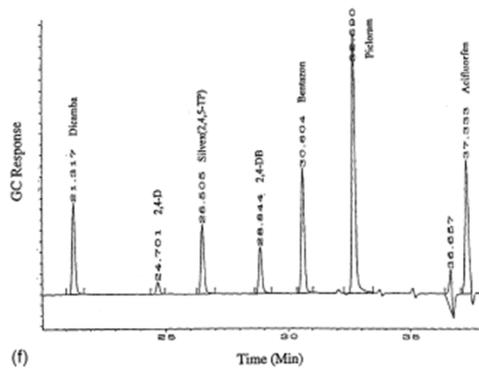
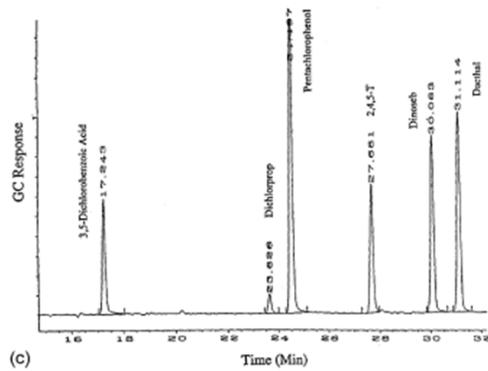
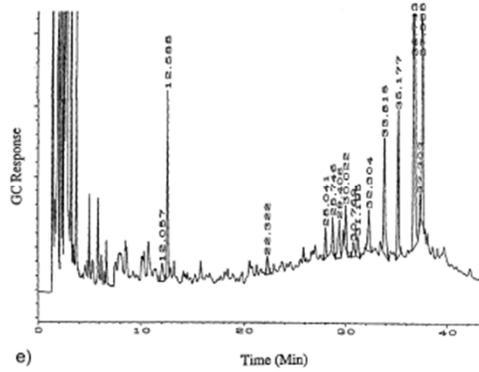
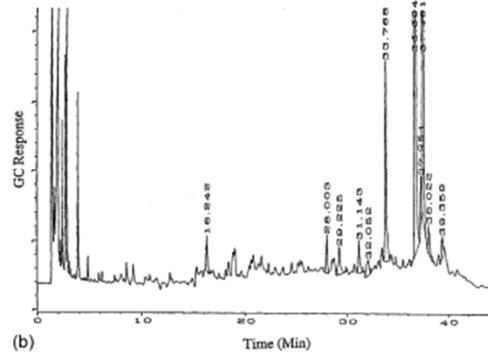
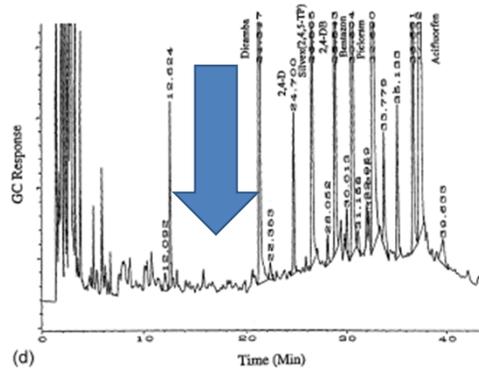
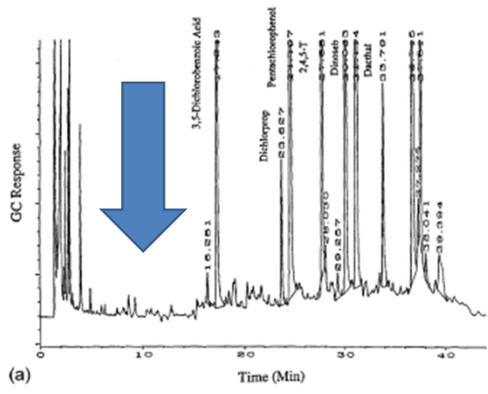
Trying to be all things to all analytes at all concentrations

## Chromatogram of a Standard



US EPA Method 625.1—Base/Neutrals and Acids  
by GC/MS ; December 2014

Figure 2 Chromatogram of combined acid/base/neutral standard



## Solid-phase Extraction—Solid-Phase Derivatization

Electronic subtraction of blanks to remove GC interferences

Yu, L.Z. and M.J.M. Wells\*. 2007. Establishing the Feasibility of Coupled Solid-phase Extraction—Solid-Phase Derivatization for Acidic Herbicides. *J. Chromatogr. A*, 1143:16-25.

## Calculated Recovery

**Matrix effects on Mass Spectrometric detectors especially in LC necessitate the use of internal standards (LC-MS/MS).**

**Don't be fooled. Are SPE results reported according to:**

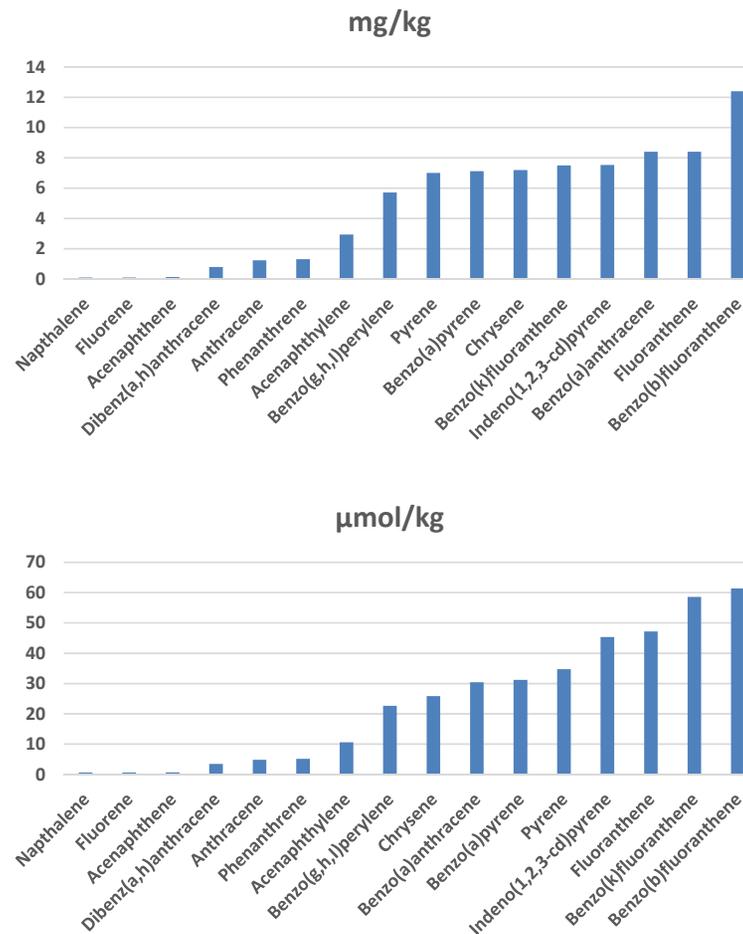
- **Absolute recovery?**
- **Recovery relative to internal standards?**

## Reporting the Data

	mg/kg	μmol/kg
Acenaphthene	0.13	0.72
Acenaphthylene	2.94	10.65
Anthracene	1.23	4.88
Benzo(a)anthracene	8.40	30.43
Benzo(a)pyrene	7.12	31.23
Benzo(b)fluoranthene	12.40	61.39
Benzo(g,h,i)perylene	5.71	22.66
Benzo(k)fluoranthene	7.50	58.59
Chrysene	7.19	25.86
Dibenz(a,h)anthracene	0.79	3.48
Fluoranthene	8.40	47.19
Fluorene	0.10	0.66
Indeno(1,2,3-cd)pyrene	7.53	45.36
Napthalene	0.10	0.66
Phenanthrene	1.31	5.20
Pyrene	7.01	34.70
<b>Σ</b>	<b>77.86</b>	<b>383.67</b>

**Moles Matter!!!**

## PAHs in soil at a Superfund site



## Reporting the Data

	mg/kg	μmol/kg
Acenaphthene	0.13	0.72
Acenaphthylene	2.94	10.65
Anthracene	1.23	4.88
Benzo(a)anthracene	8.40	30.43
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Indeno(1,2,3-cd)pyrene	7.53	45.36
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Phenanthrene	1.31	5.20
Pyrene	7.01	34.70
<b>Σ</b>	<b>77.86</b>	<b>383.67</b>

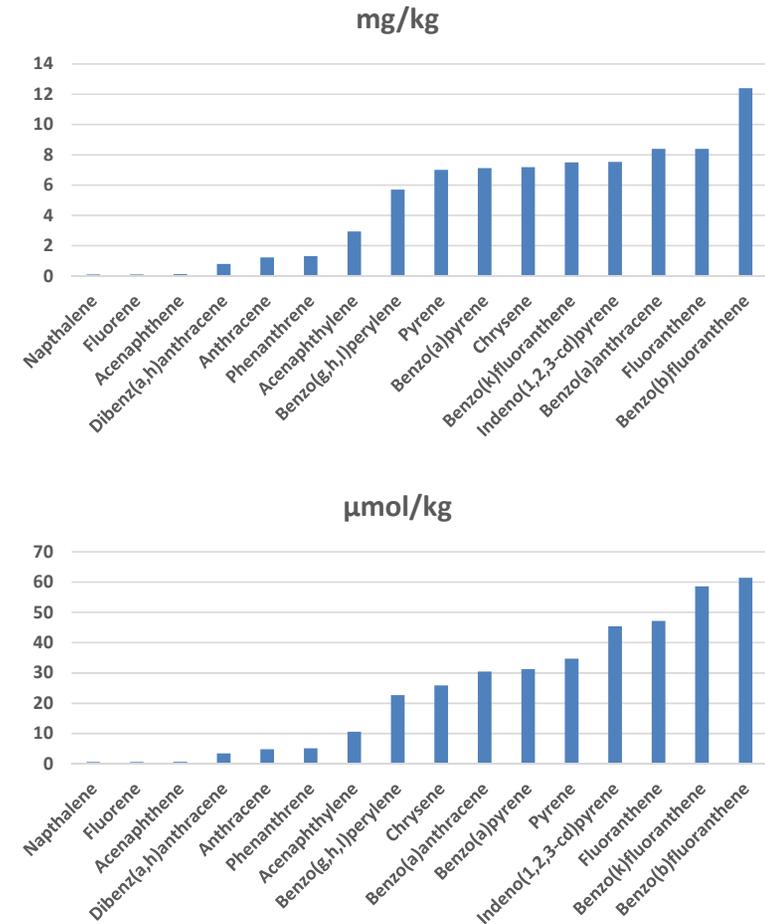
Molar concentrations versus  
Mass concentrations



**Moles Matter!!!**

Professor Molenium (on left)

## PAHs in soil at a Superfund site



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Cookeville, TN 38505

<http://scholar.google.com/citations?user=nGH9HBkAAAAJ>

### Solid-Phase Extraction (SPE) Publications

#### Refereed Articles (corresponding author denoted by an asterisk)

Boles, T.H. and M.J.M. Wells\*. 2016. Analysis of amphetamine and methamphetamine in municipal wastewater influent and effluent using weak cation-exchange solid-phase extraction (SPE) and liquid chromatography tandem mass spectrometry (LC-MS/MS). *Electrophoresis*, 37(23-24):3101-3108.

Boles, T.H. and M.J.M. Wells\*. 2014. Pilot Survey of Methamphetamine in Sewers using a Polar Organic Chemical Integrative Sampler. *Science of the Total Environment*, 472: 9-12.

Sunkara, M. and M.J.M. Wells\*. 2010. Phase II Pharmaceutical Metabolites Acetaminophen Glucuronide and Acetaminophen Sulfate in Wastewater. *Environ. Chem.*, 7:111-122.

Yu, L.Z. and M.J.M. Wells\*. 2007. Establishing the Feasibility of Coupled Solid-phase Extraction—Solid-Phase Derivatization for Acidic Herbicides. *J. Chromatogr. A*, 1143:16-25.

Sutherland, D.J., G.K. Stearnman, and M.J.M. Wells\*. 2003. Development of an Analytical Scheme for Simazine and 2,4-D in Soil and Water Runoff from Ornamental Plant Nursery Plots. *J. Agric. Food Chem.*, 51:14-20.

Garimella, U.I., G.K. Stearnman, and M.J.M. Wells\*. 2000. Comparison among Soil Series and Extraction Methods for the Analysis of Trifluralin. *J. Agric. Food Chem.*, 48:5874-5880.

Wells, M.J.M.\* and L. Zhou Yu. 2000. Solid-Phase Extraction of Acidic Herbicides. *J. Chromatogr. A*, 885:237-250.

Wells, M.J.M.\*, D.M. Ferguson, and J.C. Green. 1995. Determination of Oil and Grease in Waste Water by Solid-Phase Extraction. *Analyst*, 120:1715-1721.

Wells, M.J.M.\*, A.J. Rossano, Jr., and E.C. Roberts. 1994. Textile Wastewater Effluent Toxicity Identification Evaluation. *Arch. Environ. Contam. Toxicol.* 27(4):555-560.

Wells, M.J.M.\*, D.D. Riemer, and M.C. Wells-Knecht. 1994. Development and Optimization of a Solid-Phase Extraction Scheme for Determination of the Pesticides Metribuzin, Atrazine, Metolachlor and Esfenvalerate in Agricultural Runoff Water. *J. Chromatogr. A*. 659:337-348.

Wells, M.J.M.\*, A.J. Rossano, Jr., and E.C. Roberts. 1990. Solid-Phase Extraction for Toxicity Reduction Evaluations. *Anal. Chim. Acta* 236:131-139.

Wells, M.J.M.\* and J.L. Michael. 1987. Recovery of Picloram and 2,4-Dichlorophenoxyacetic Acid from Aqueous Samples by Reversed-Phase Solid-Phase Extraction. *Anal. Chem.* 59:1739-1742.

Wells, M.J.M.\* and J.L. Michael. 1987. Reversed-Phase Solid-Phase Extraction for Aqueous Environmental Sample Preparation in Herbicide Residue Analysis. *J. Chromatogr. Sci.* 25:345-350.

Wells, M.J.M.\*, J.L. Michael, and D.G. Neary. 1984. Determination of Picloram in Soil and Water by Reversed-Phase Liquid Chromatography. *Arch. Environ. Contam. Toxicol.* 13:231-235.

Wells, M.J.M.\* 1982. The Effect of Silanol Masking on the Recovery of Picloram and Other Solutes from a Hydrocarbonaceous Pre-Analysis Extraction Column. *J. Liquid Chromatogr.* 5:2293-2309.

#### Technical Reports and Non-Refereed Publications (corresponding author denoted by an asterisk)

Wells, M.J.M.\* and N. Simpson. 1992. Determination of Oil and Grease in Wastewater. *Varian Instrument Applications (VIA)* 21(1):10.

Roberts, E.C.\*, A.J. Rossano, Jr., and M.J.M. Wells. 1990. Approaches to the Identification of Potential Toxicants in Textile Wastewater Effluents. *AATCC Symposium on Safety, Health, and Environmental Technology - Book of Papers No. 45*, pp. 10-18.

Wells, M.J.M.\* 1990. Applications of Solid-Phase Extraction to Environmental Samples. *Tennessee Directory of Natural Resources Research - Water Quality and Soil and Water Conservation Projects*. Tennessee Soil and Water Research and Education Committee, p. 29.

Wells, M.J.M.\* 1986. Off-Line Multistage Extraction Chromatography for Ultrasensitive Herbicide Residue Isolation. In: *Proceedings of the Third Annual International Symposium on Sample Preparation and Isolation Using Bonded Silicas*. Analytichem International, Harbor City, CA, pp. 117-135.

Wells, M.J.M.\* 1985. General Procedures for the Development of Adsorption Trapping Methods Used in Herbicide Residue Analysis. In: *Proceedings of the Second Annual International Symposium on Sample Preparation and Isolation Using Bonded Silicas*. Analytichem International, Harbor City, CA, pp. 63-68.

#### Book Chapters (corresponding author denoted by an asterisk)

Wells, M.J.M.\* 2003. Principles of Extraction and the Extraction of Semivolatile Organics from Liquids. In: *Sample Preparation Techniques in Analytical Chemistry*, S. Mitra, ed., John Wiley & Sons, pp. 37-138.

Wells, M.J.M.\* 2000. Essential Guides to Method Development in Solid-phase Extraction. In: *Encyclopedia of Separation Science*. I.D. Wilson, E.R. Adlard, M. Cooke, and C.F. Poole, Eds., Volume 10:4636-4643. Academic Press Ltd, London, UK.

Simpson, N.\* and M.J.M. Wells. 2000. Introduction to Solid-phase Extraction. In: *Solid-phase Extraction: Principles, Techniques, and Applications*. N. Simpson, Ed., Marcel Dekker, Inc., New York, NY, pp. 1-17.

Wells, M.J.M.\* 2000. Handling Large Volume Samples: Applications of SPE to Environmental Matrices. In: *Solid-phase Extraction: Principles, Techniques, and Applications*. N. Simpson, Ed., Marcel Dekker, Inc., New York, NY, 97-123.

Wells, M.J.M.\* and G. K. Stearnman. 1996. Coordinating Supercritical Fluid and Solid-Phase Extraction with Chromatographic and Immunoassay Analysis of Herbicides. In: *Herbicide Metabolites in Surface Water and Groundwater*. M.T. Meyer and E.M. Thurman, Eds., ACS Symposium Series No. 630, American Chemical Society, Washington, DC, pp.18-33.

# Acknowledgments

I gratefully acknowledge the numerous students and colleagues who have collaborated with me over four decades to accomplish SPE research objectives.

You enhanced scientific inquiry and enriched my life.

Thanks to the symposium organizers for inviting me to share my story.

---

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