New Advances in the Automation of Cartridge Based Solid Phase Extraction for Supporting EPA 500 Series Methods

Michael Ebitson: Horizon Technology

Advancement Goals: Automated Extractor

- “Chemistry- first” design approach
- Create a small but powerful instrument that can process a wide variety of sample size.
- Implement positive pressure for improved sample delivering and precise solvent volumes.
- Unattended and worry free operation.
- Modular and scaleable design for maximum throughput
- Ensure multiple methods could be used with different size cartridges.

Theory of Operation

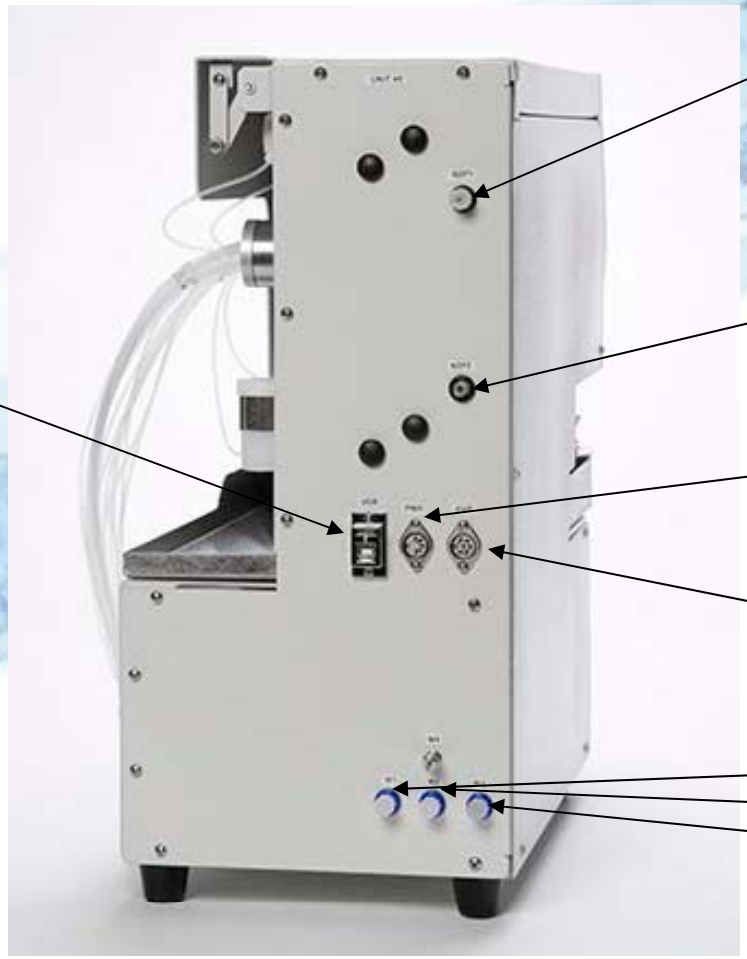
Automation achieves a higher level of consistent recoveries by controlling all critical extraction times and parameters.

The Extraction Cycle—A Detailed Look

1. Condition the SPE cartridge with reagents.
2. Introduce the sample to the SPE cartridge.
3. Wash the SPE cartridge with reagent water.
4. Dry the SPE cartridge with nitrogen gas (to remove residual water from the packing).
5. Elute the SPE cartridge with the eluting reagents

SmartPrep Hardware Overview

Back Panel Features



N2P1 – Low Pressure Nitrogen Gas Inlet Fitting

N2P2 – High Pressure Nitrogen Gas Inlet Fitting

USB Comm Port

Power: 24 VDC Power Receptacle

Expansion: Future Comm Port

Waste Port Fittings W1, W2, W3 (user definable)

Advanced Fluid Handing

- Accurate and reproducible volume delivery of the desired reagent's.
- Able to Handle of a full range of reagents.
- Rinsing the sample container with the eluting reagent.
- Accurately and reproducibly mix reagent gradients.
- To minimize, or eliminate residual air from being introduced into a previously conditioned cartridge.
- Monitoring the presence of all reagents, so the cartridge is never allowed to inadvertently go dry.
- Delivery of all liquids (reagents and samples) directly to the top of the packing material and not simply into the body of the cartridge barrel.
- Purging all parts and lines with an inert gas.
- Compensating for reagent and sample out gassing.
- Cleaning all flow paths to eliminate carryover and background contamination.

Reagent Management Assist (RMA)

- Internal Liquid Sensor:
  - Volume verifier for both solvent and sample intake and displacement.
  - Will auto calculate the sample volume and store it in the time stamped report
- The RMA allows you to have live solvent volume status reporting during and not during use.
  - Enables solvent volume levels to me monitored easily.
- The RMA displays a solvent volume calculator
  - This calculates how much solvent will be consumed and prompts the operator to ensure that enough solvent is available prior to the start of each run.

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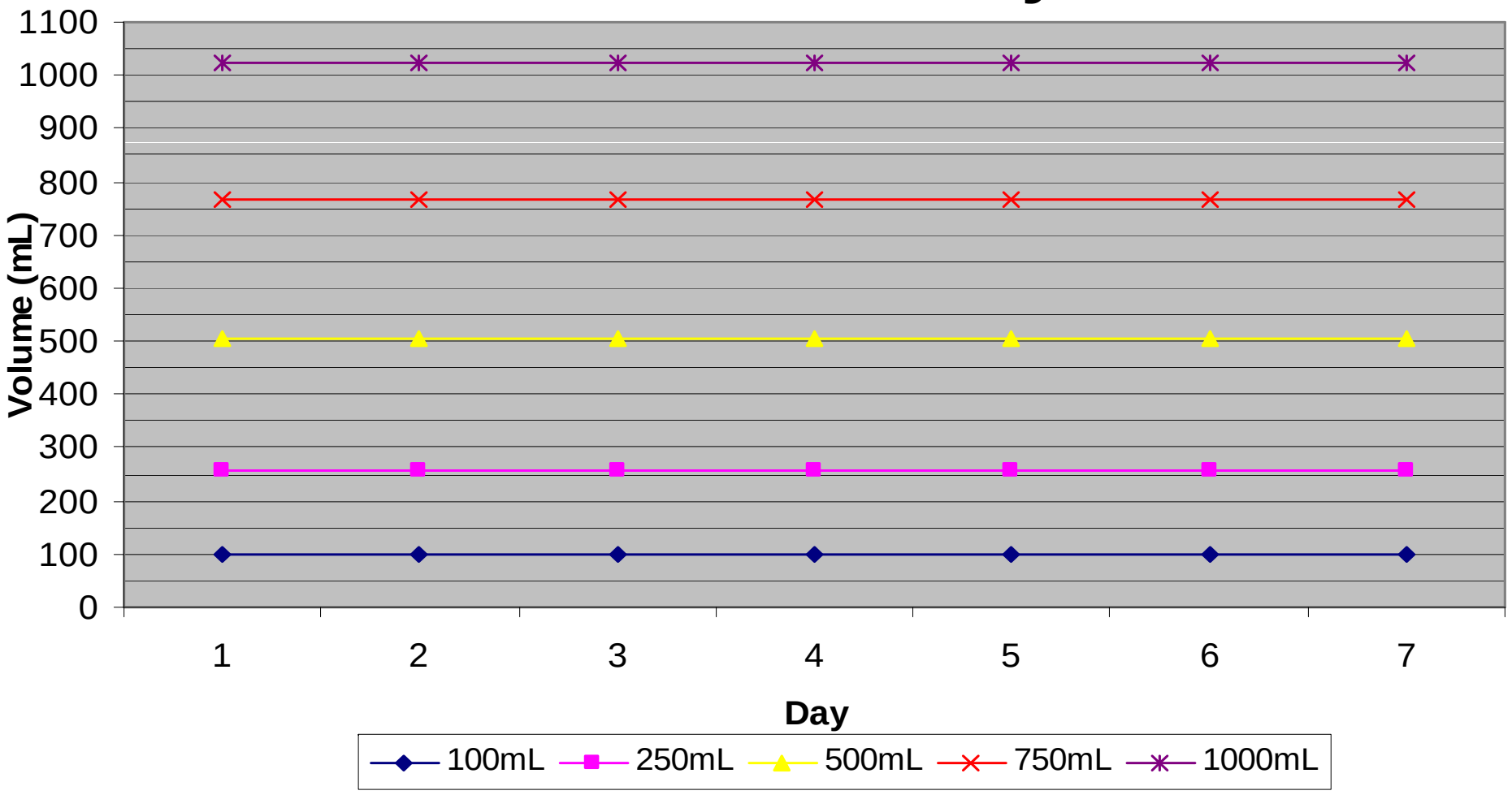
Automatic Sample Volume Calculation Study



Automatic Sample Volume Calculation

- A total of 7 replicates were run over a 7 day period for five different amounts of water

Replicate	Actual Volume (g)	Extimated Volume (mL)	Error (%)
1	100	100	0.0
2	250	256	2.3
3	500	507	1.4
4	750	765	2.0
5	1000	1022	2.2

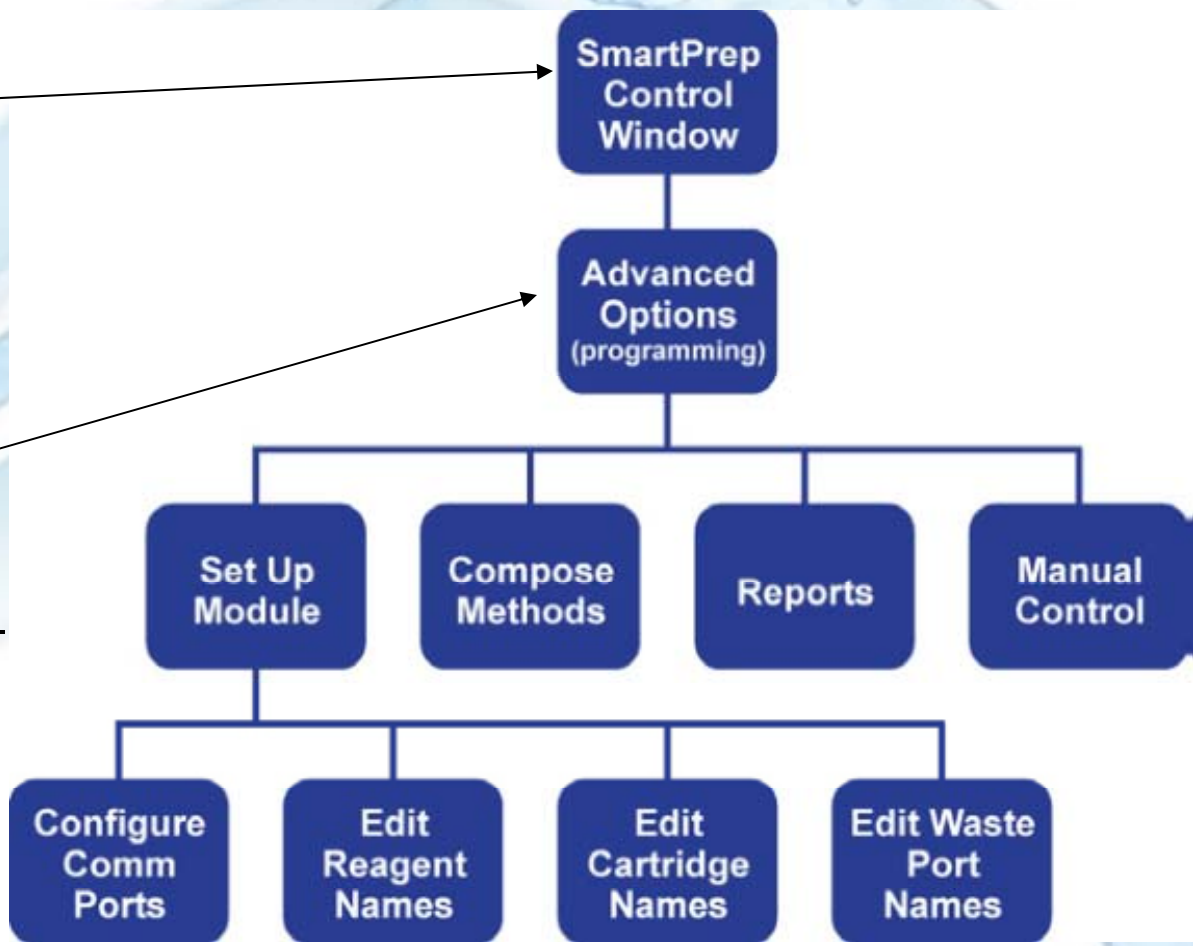
This automated feature eliminates the subjective process of the operator marking and refilling a sample bottle, and transferring to a graduated cylinder and replaces it with an absolute process containing less error.

Software Overview

The SmartPrep software has two main sections:

- The **SmartPrep Control** window (described in the remainder of this section), where you load methods and run the various modules.

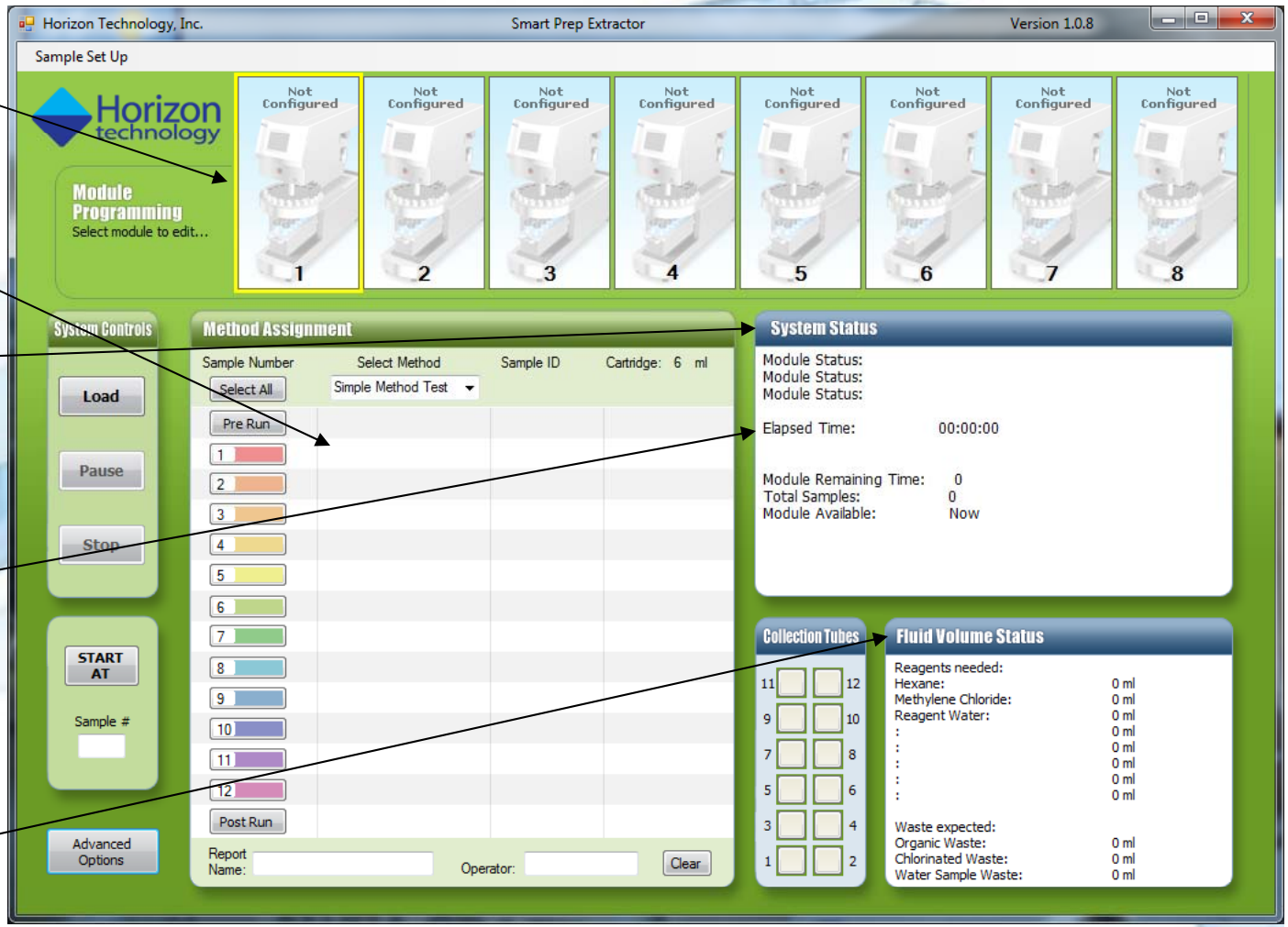
- The **Advanced Options** (programming) windows, where you can configure modules, compose methods, generate reports, and permit manual control. You can password-protect the Advanced Options windows to prevent unauthorized access to critical methods and settings.



Control Window: Graphical User Interface

The SmartPrep Control Window Displays

- Total number of modules connected to the PC Controller.
- Specific methods being run.
- The current sample and step within a method being processed.
- The total time elapsed and remaining to complete the samples.
- The total volume of reagents used and the volume of generated waste.



Advanced Options: Set Up Module

The **Set Up Module** button accesses features to properly configure a module. When you click this button, the System Configuration window is displayed

The System Configuration Window

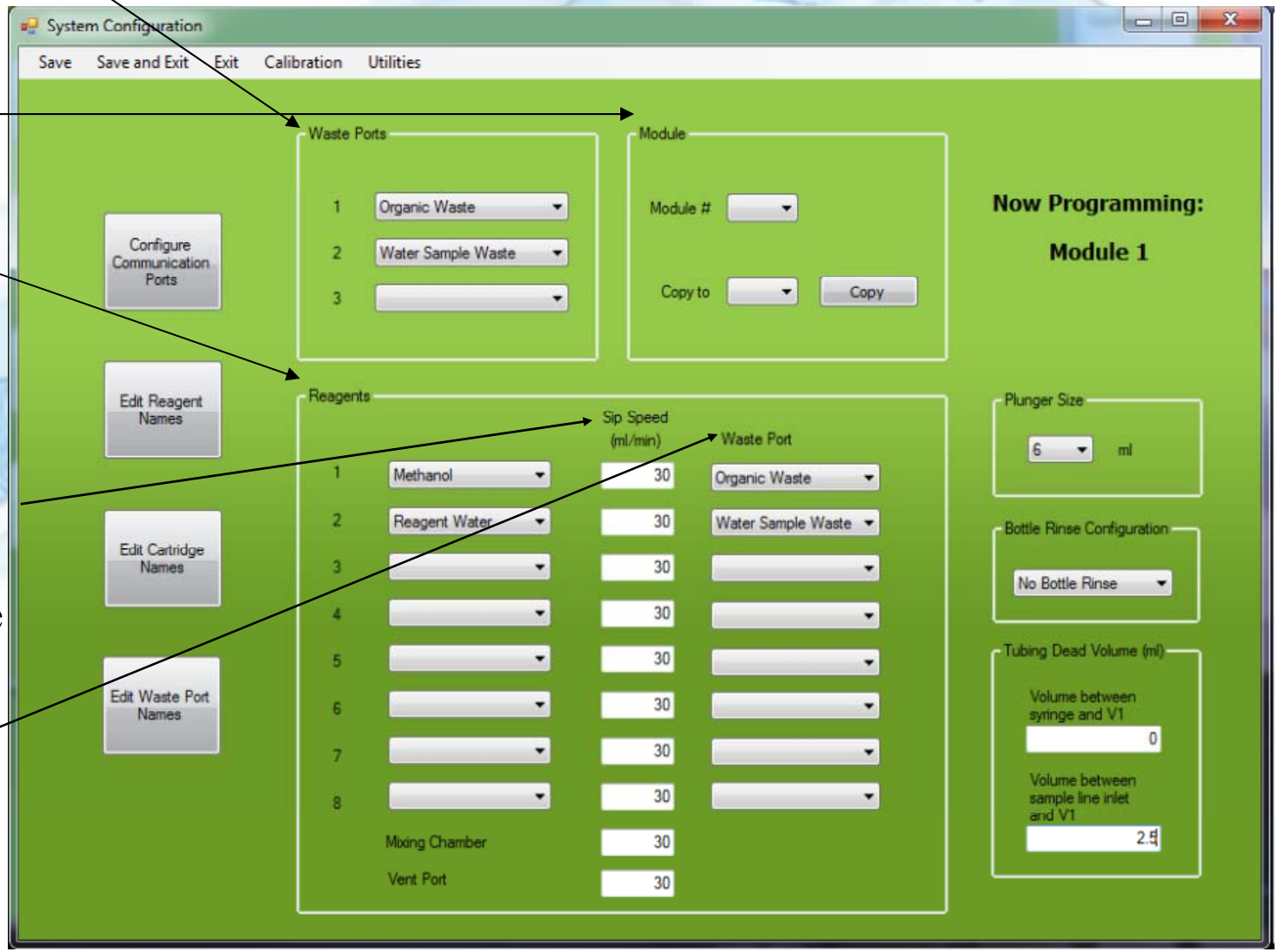
Waste Port selection

You can connect and control up to 8 SmartPrep **Modules** from a single PC.

Reagents, you must make three selections:

- The reagents to be used (Up to 50 may be entered)
- **Sip speed**-the speed at which the reagent will be pulled from the reagent container.

- The **waste ports** to which the reagents should be directed .

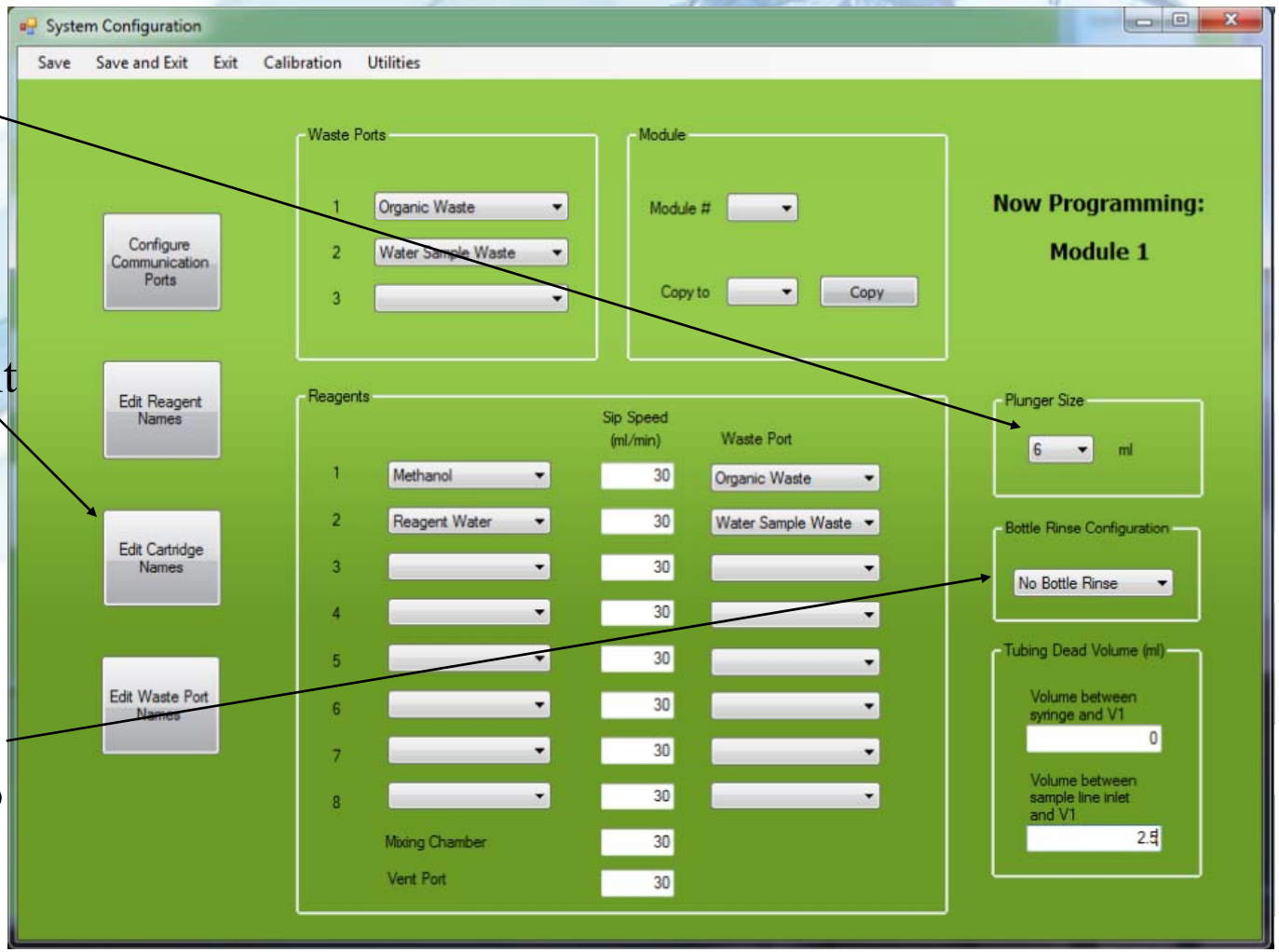


The System Configuration Window

If a 1 or 3 mL cartridge is desired, select the **plunger size** using the drop down arrow (Only 1 size cartridge may be used run at a time)

If unique cartridges are desired, up to 40 different cartridges many be entered

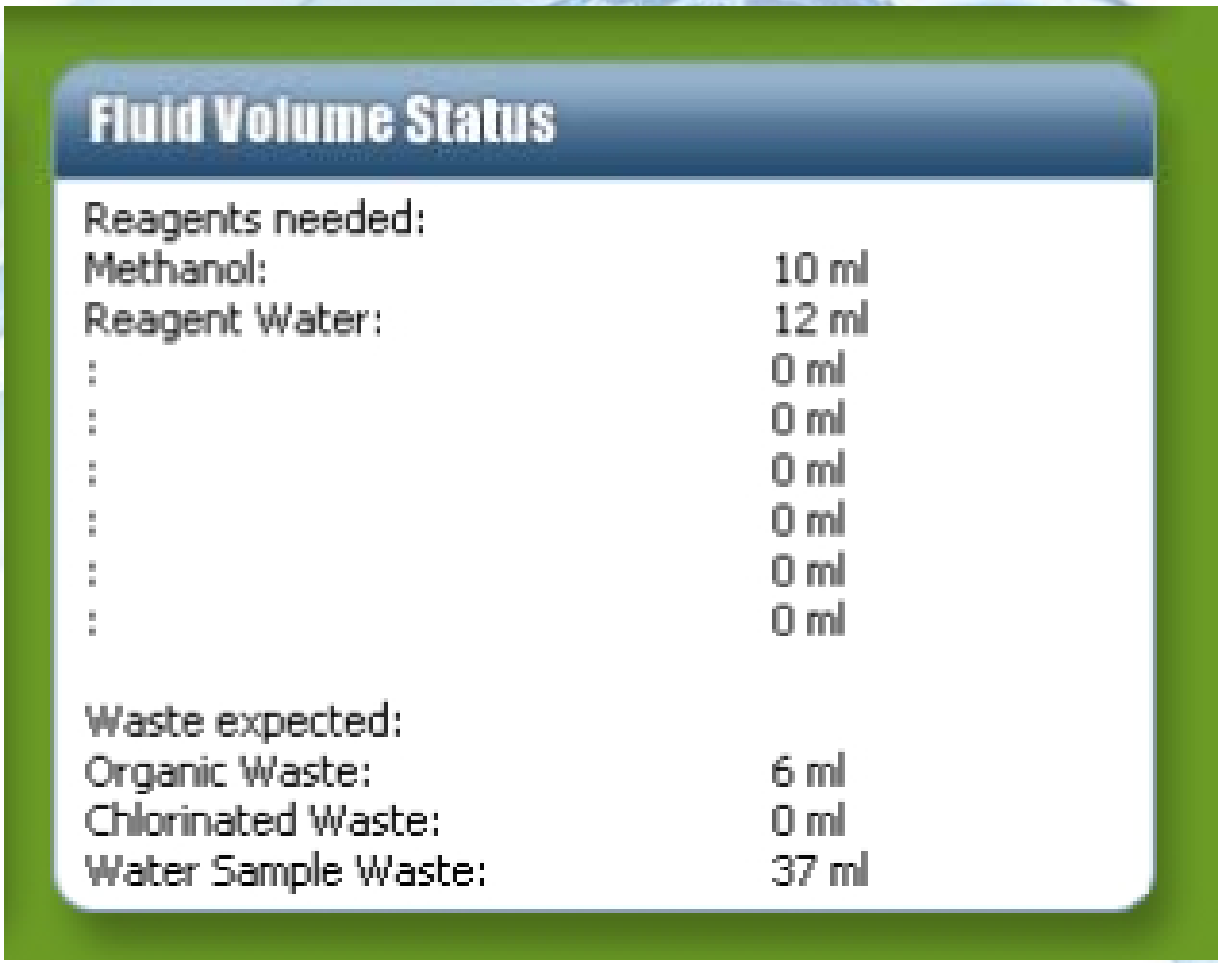
Each module can be equipped with the optional Reagent Rinse Bottle Kit. If the Kit is to be used, select this **Yes Bottle Rinse**



Fluid Volume Status Panel

When a method is assigned to a sample, the software automatically calculates the **total volume of reagents required**, and the **total volume of liquid waste** that will be generated. The waste volume also includes the **total volume of water sample** that will be processed.

This information is very useful and should be checked before starting the module to ensure sufficient volumes of reagents are available and that all waste containers are empty, and of the proper capacity to handle waste volume.

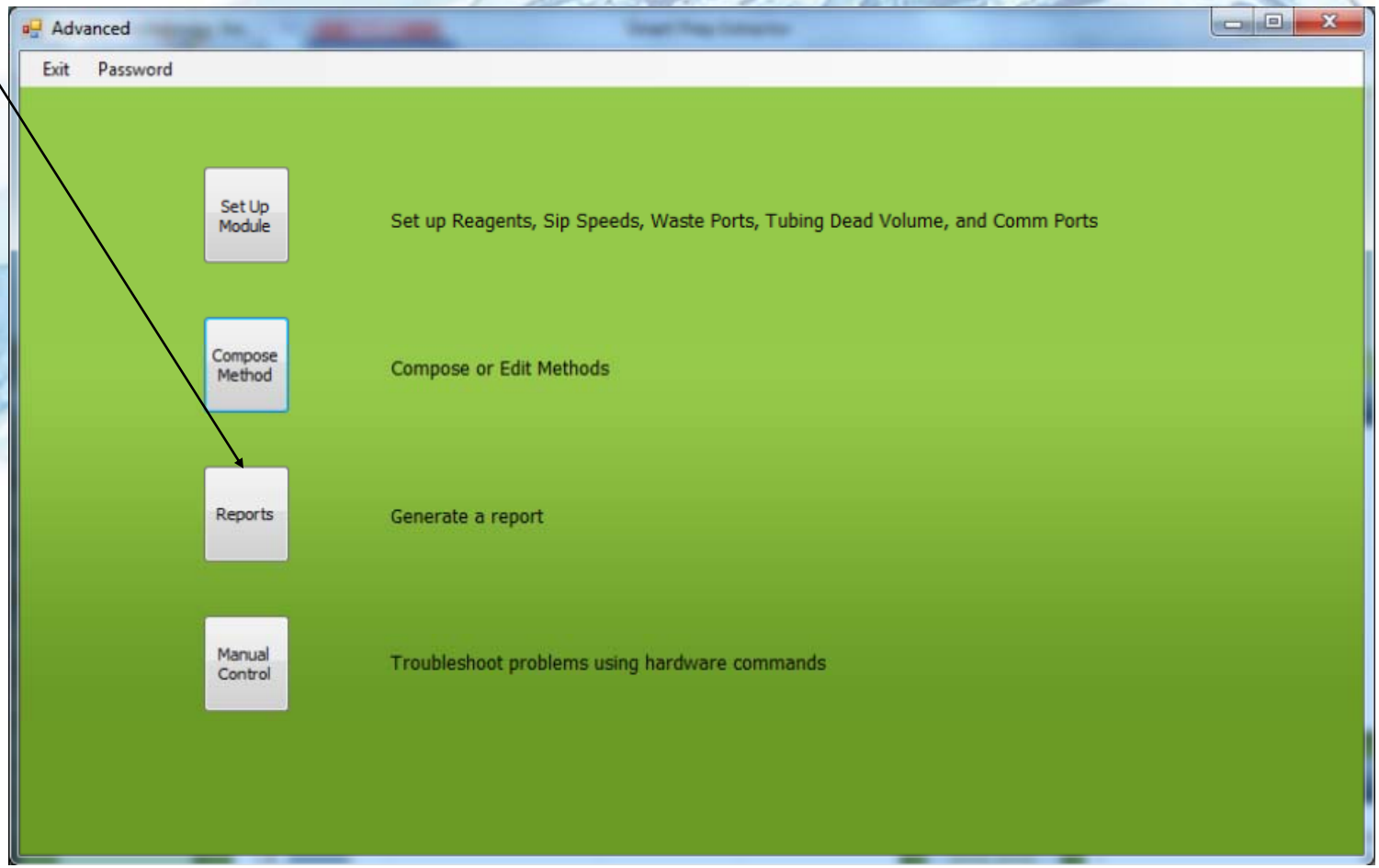


Generating Time Stamped Audit Trails

The **Reports** button accesses features to view and print any run report files.

When you click this button, the View and Print Reports window is displayed:

This allows for LIMS interactivity



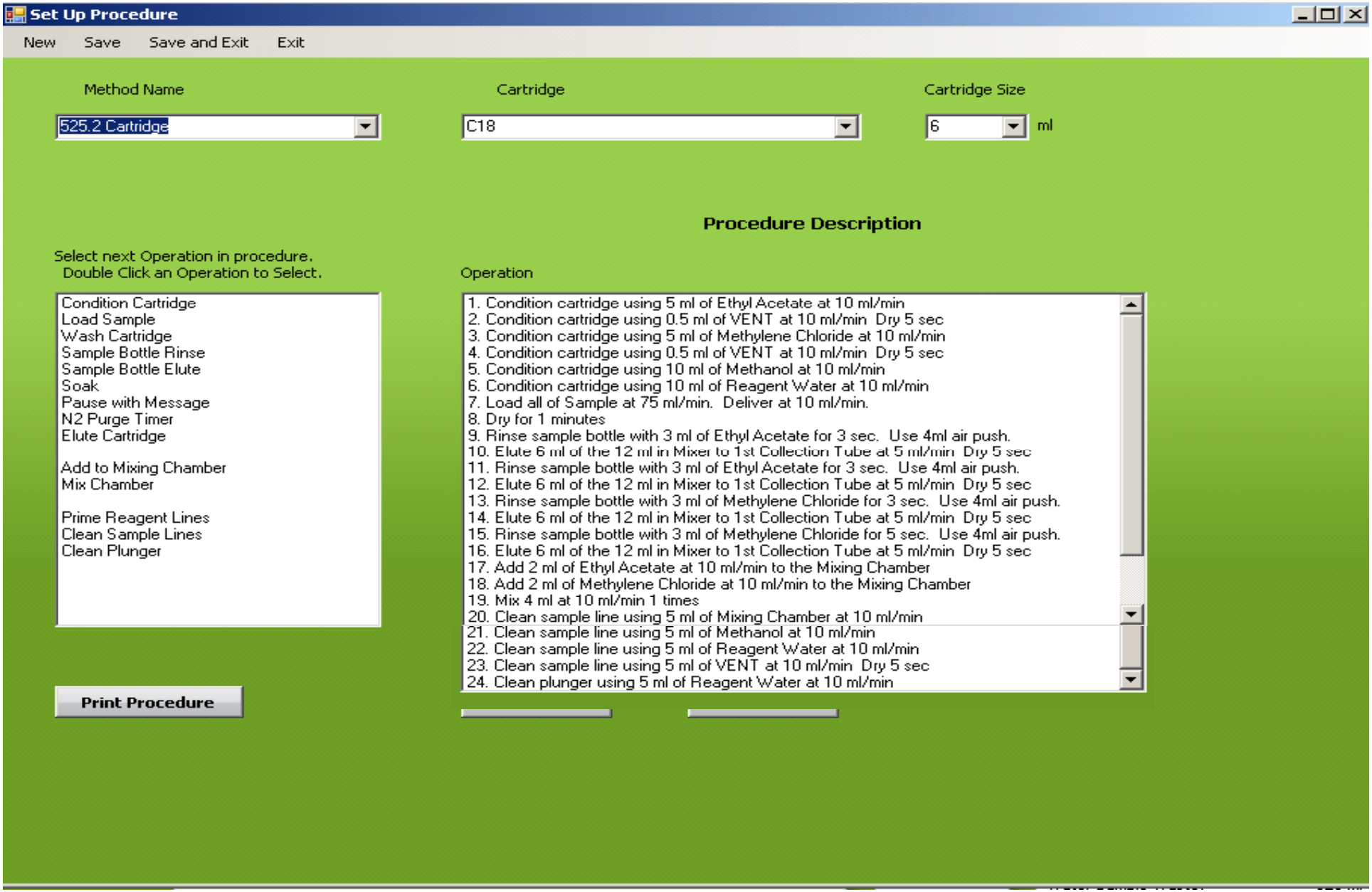
Environmental SPE Methods

- 500 series
  - (Drinking Water) The EPA 500 methods are designed to identify and quantify organic compounds in municipal drinking water. These methods are cited under the Safe Drinking Water Act (SDWA).
- 600 series
  - (Waste Water) The EPA 600 methods are designed for monitoring organic pollutants in industrial and municipal waste discharges. These methods are cited under the Clean Water Act (CWA).
- 1600 series
  - (Waste Water) The EPA 1600 methods designed for monitoring Wastewater Pollutants
- 8000 series
  - (Ground Water) The EPA 8000 methods are designed for monitoring organic pollutants in ground water, as prescribed in the Resource Recovery and Conservation Act (RCRA).

Environmental SPE Methods

EPA Method	CLASS OF COMPOUNDS	Cartridge Sorbent
521	Nitrosamines	2 g Coconut Charcoal
522	1,4-Dioxane	Opt 1: 2 g Coconut Charcoal
528	Phenols	S/DVB
535	Chloroacetanilide and other Acetamide Herbicide Degradates	0.5 g NP Graphitized Carbon
537	Perfluorinated alkyl acids	S/DVB
548.1	Endothall	tertiary amine anion exchange
506	Phthalate and Adipate Esters	C18
508.1	Pesticides, Herbicides, and Organohalides	C18
515.2/3	Chlorinated Acids	Resin Based
525.2	Organic Compounds	C18
526	Selected Semivolatile Organic Compounds	S/DVB
532	Phenylurea Compounds	C18
549.2	Paraquat and Diquat	C8
550.1	PAH's	C18
552.1	Haloacetic Acids and Dalapon	AG-1-X8 anion exchange resin

EPA Method 525.2



EPA Method 525.2 Results IDP Study (0.5ppb)

525.2 Compounds	Average % Recovery	%RSD
Acenaphthene d10	70.30	5.52
Phenanthrene d10	75.45	3.40
Chrysene d12	82.10	9.43
Isophorone	85.60	8.40
2-Nitro-m-xylene	83.75	9.10
Naphthalene	79.05	10.84
Dichlorvos	109.75	5.55
EPTC	105.00	3.24
Mevinphos	118.15	4.20
Butylate	106.90	1.99
Vernolate	108.15	1.68
Dimethyl phthalate	117.95	2.31
Pebulate	109.85	1.03
Etridiazole	110.60	2.59
2,6-Dinitrotoluene	83.80	9.49
Acenaphthylene	102.80	1.38
Chloroneb	120.05	1.86
Tebuthiuron	125.85	9.04
2,4-Dinitrotoluene	85.10	11.06
Molinate	113.40	1.54
Diethyl phthalate	124.10	4.69
Fluorene	110.15	2.83
Propachlor	122.00	6.38
Ethoprop	124.50	7.54
Cycloate	116.55	4.38
Chlorpropham	125.10	7.89
Trifluralin	114.75	2.64
a-BHC	112.35	1.63
Atraton	95.05	7.89
Hexachlorobenzene	93.70	5.46
Prometon	113.90	1.77
Lindane (g-BHC)	116.20	1.55
Simazine	116.75	1.15
Atrazine	117.40	1.82
Propazine	114.95	0.43
b-BHC	114.80	1.36
Pentachlorophenol	122.55	4.49

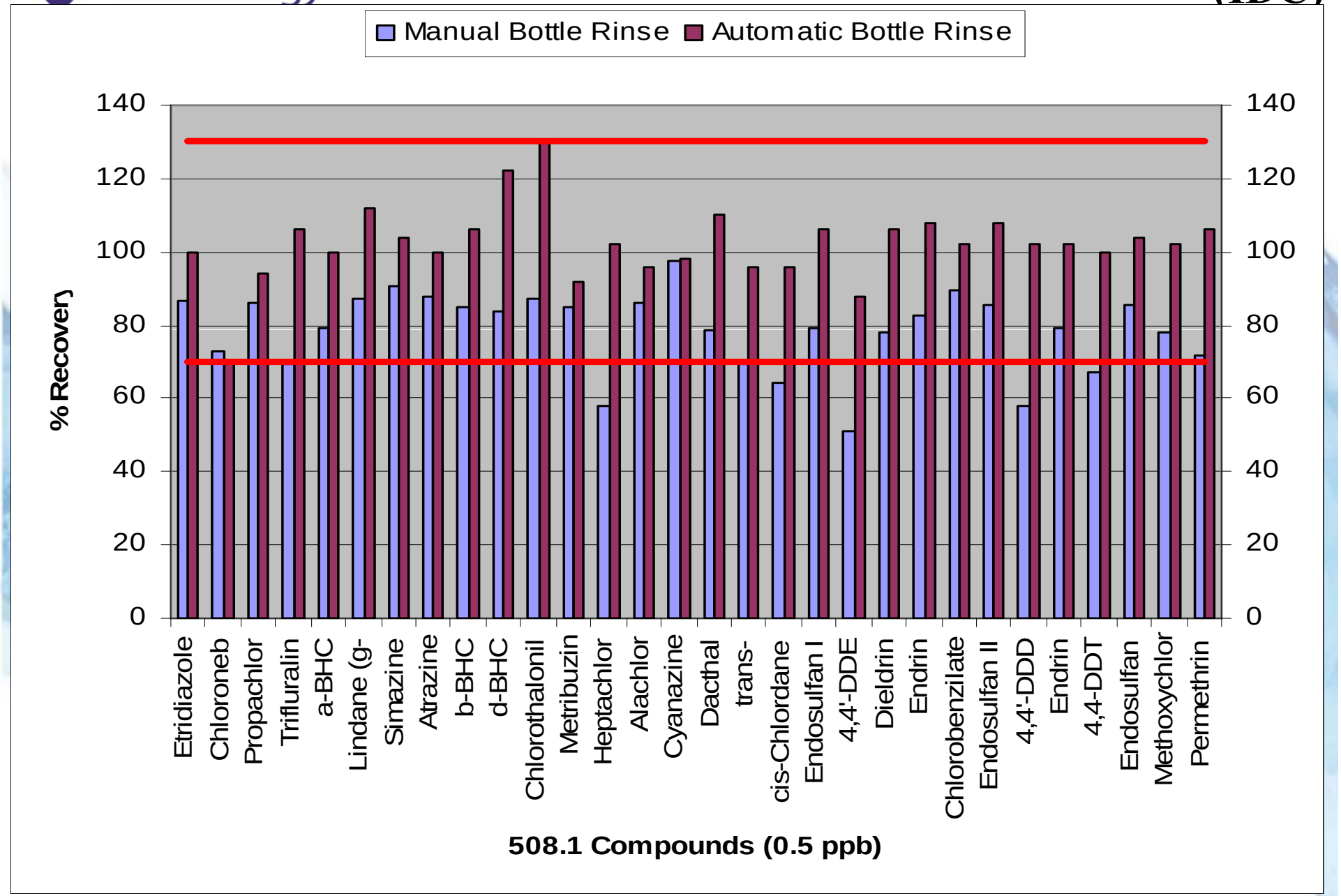
525.2 Compounds	Average % Recovery	%RSD
Terbufos	107.00	4.30
Pronamide	117.60	1.42
Diazinon	72.70	7.70
d-BHC	120.85	1.18
Phenanthrene	105.40	0.46
Disulfoton	108.05	2.18
Methyl paraoxon	119.25	3.06
Anthracene	103.35	0.73
Terbacil	122.15	2.76
Chlorothalonil	128.30	1.42
Metribuzin	112.70	8.77
Simetryn	114.55	1.62
Heptachlor	100.10	2.43
Ametryn	119.45	0.42
Alachlor	115.75	1.25
Prometryn	117.05	1.40
Terbutryn	115.50	0.41
Di-n-butyl phthalate	115.50	1.12
Bromacil	121.55	2.76
Cyanazine	118.45	2.16
Metolachlor	116.55	1.20
Chlorpyrifos	112.35	3.14
Aldrin	99.80	3.62
Triademefon	113.65	2.29
Dacthal	116.50	1.10
MGK-264-A	113.85	2.00
Diphenamid	115.10	3.35
MGK-264-B	123.45	1.77
Heptachlor epoxide B	111.50	3.47
Heptachlor epoxide A	110.50	4.35
Fluoranthene	104.25	0.72
g-Chlordane	97.30	6.59
Stirofos	112.50	6.44
Disulfoton sulfone	111.40	5.19
Butaclor	105.60	6.33
a-Chlordane	97.85	7.51
Endosulfan I	100.50	6.46

525.2 Compounds	Average % Recovery	%RSD
Fenamiphos	115.45	6.19
Pyrene-d10	94.55	5.48
Pyrene	95.60	5.37
Napropamide	107.45	5.91
trans-Nonachlor	96.95	5.99
4,4'-DDE	94.35	6.24
Dieldrin	100.65	6.02
Tricyclazole	116.30	5.11
Terphenyl-d14	122.60	9.45
Carboxin	106.75	5.84
Endrin	107.85	2.60
Chlorobenzilate	106.85	4.53
Endosulfan II	105.05	5.45
4,4'-DDD	98.10	5.12
Endrin Aldehyde	116.55	0.78
Butyl benzyl phthalate	106.15	3.26
Norflurazon	119.50	2.02
4,4-DDT	102.75	5.60
Endosulfan Sulfate	113.60	4.00
Bis(2-ethylhexyl)adipate	99.25	6.16
Hexazinone	116.75	2.24
Triphenylphosphate	114.10	2.17
Endrin Ketone	111.10	3.98
Methoxychlor	109.75	2.95
Benz(a)anthracene	97.80	2.23
Chrysene	100.75	1.72
Bis(2-ethylhexyl)phthalate	109.55	2.90
Fenarimol	120.30	3.91
cis-Permethrin	105.65	4.47
trans-Permethrin	103.30	4.39
Di-n-octyl phthalate	106.00	5.09
Benzo(b)fluoranthene	98.35	4.89
Benzo(k)fluoranthene	100.50	4.24
Benzo(a)pyrene	97.70	3.12
Fluridone	127.70	6.73
Perylene-d12	94.75	4.21
Indeno(1,2,3-cd)pyrene	99.35	5.42
Dibenz(ah)anthracene	96.45	4.00
Benzo(ghi)perylene	100.05	5.06

Extraction Method: EPA 508

Step	Conditioning	Reagent	Volume (mL)	Rate (mL/min)	Soak (s)	N2 Purge (s)	Liquid Sense	
1		EtOAc	5	10	10	2	No	
2		DCM	5	10	10	2	No	
3		DCM	5	10	10	2	No	
4		MeOH	5	10	0	0	No	
5		MeOH	5	10	10	0	No	
6		DI H20	5	10	0	0	No	
7		DI H20	5	10	10	0	No	
8	Load Sample	Load Until Empty (mL)	Syringe Fill Pause (s)	Sample Sip Rate (mL/min)	Sample Deliver Rate (mL/min)	Minimum Volume (mL)	Expected Volume (mL)	
		Yes	1	20	10	800	1000	
9	N2 Purge Timer	Delay (min)						
		5						
10	Sample Bottle Rinse	Reagent	Volume (mL)	Vent Volume (mL)	Spray Time (s)	Liquid Sense		
		EtOAc	5	5	5	No		
11	Sample Bottle Elute	Volume (mL, to Mixer)	Destination (Tube)	Volume (mL, to elute)	Sip Rate (mL/min)	Delivery Rate (mL/min	Soak (s)	N2 Purge (s)
		15	1	5	10	10	20	1
12	Sample Bottle Rinse	Reagent	Volume (mL)	Vent Volume (mL)	Spray Time (s)	Liquid Sense		
		DCM	5	5	5	No		
13	Sample Bottle Elute	Volume (mL, to Mixer)	Destination (Tube)	Volume (mL, to elute)	Sip Rate (mL/min)	Delivery Rate (mL/min	Soak (s)	N2 Purge (s)
		10	1	5	10	10	20	1
14	Sample Bottle Rinse	Reagent	Volume (mL)	Vent Volume (mL)	Spray Time (s)	Liquid Sense		
		DCM	5	5	5	No		
15	Sample Bottle Elute	Volume (mL, to Mixer)	Destination (Tube)	Volume (mL, to elute)	Sip Rate (mL/min)	Delivery Rate (mL/min	Soak (s)	N2 Purge (s)
		10	1	5	10	10	20	1
16	Add to Mixing Chamber	Reagent	Volume (mL)	Rate (mL/min)				
		DCM	5	10				
17	Elute Cartridge	Reagent	Volume (mL)	Rate (mL/min)	Destination (Tube)	Soak (s)	Purge (s)	
		Mixing Chamber	10	10	1	5	15	

EPA Method 508.1:Sample Bottle Rinsing Study (IDC)



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Phenomenex
- Strata® C18-E, 6 mL Cartridges

- Zebron™ – Multiresidue 1:- 30 m x 0.32 mm x 0.50 µm

- Zebron™ - Multiresidue 2:- 30 m x 0.25 mm x 0.25 µm

Results EPA 508: 12 Replicates (0.5ug/L)

		Blanks		LCS			
		Average (ug/L) MR-1	Average (ug/L) MR-2	Average (%) MR-1	RSD (%) MR-1	Average (%) MR-2	RSD (%) MR-2
Etridiazole		0.01	0.00	115	12.64	84	8.99
Chloroneb		0.02	0.00	90	5.23	68	6.70
Propachlor		0.03	0.01	92	9.59	86	8.31
Trifluralin		0.01	0.01	83	6.11	92	10.06
a-BHC		0.00	0.00	87	5.67	94	6.89
Lindane (g-BHC)		0.00	0.02	93	5.37	104	6.91
Simazine	1	N/A	0.06	N/A	N/A	94	9.60
Atrazine	1	N/A	0.09	N/A	N/A	95	7.05
b-BHC		0.01	0.00	94	3.99	103	5.74
d-BHC	2	N/A	0.00	N/A	N/A	114	7.75
Chlorothalonil		0.00	0.00	112	8.72	117	10.13
Metribuzin	2	N/A	0.00	N/A	N/A	83	8.44
Heptachlor		0.03	0.01	91	13.83	86	13.64
Alachlor		0.05	0.00	89	11.32	92	10.18
Cyanazine		0.01	0.00	119	8.60	94	4.48
Metolachlor		0.00	N/A	100	5.37	N/A	N/A
Dacthal		0.00	0.00	92	3.75	101	6.13
Heptachlor epoxide B		0.00	0.00	89	4.22	99	6.66
trans-Chlordane		0.00	0.00	84	4.93	91	7.37
Butaclor		0.00	0.00	105	6.81	95	8.19
cis-Chlordane		0.00	0.00	85	4.62	90	6.66
Endosulfan I		0.00	0.00	89	4.01	98	6.76
4,4'-DDE		0.01	0.01	78	7.19	85	9.74
Dieldrin		0.00	0.00	94	4.54	97	6.26
Endrin		0.00	0.00	97	6.56	100	7.74
Chlorobenzilate		0.00	0.00	70	12.60	92	10.99
Endosulfan II		0.00	0.00	92	4.14	99	8.15
4,4'-DDD		0.01	0.00	91	5.00	92	8.40
Endrin Aldehyde		0.01	0.00	90	4.28	92	8.90
4,4-DDT		0.01	0.00	107	6.82	94	8.75
Endosulfan Sulfate		0.00	0.00	96	4.67	96	8.68
Methoxychlor		0.01	0.00	118	10.22	94	10.99
Permethrin		0.05	0.00	107	16.09	106	16.99
Decachlorobiphenyl	S	0.40	0.46	83	6.23	96	10.66

1, 2 - Co-Elutting compounds on MR-1

522: Extraction Method

Step	Conditioning	Reagent	Volume (mL)	Rate (mL/min)	Soak (s)	N2 Purge (s)	Liquid Sense	
1		DCM	3	20	10	2	No	
2		MeOH	3	20	10	2	No	
3		MeOH	3	20	10	0	No	
4		DI	3	20	10	0	No	
5		DI	3	20	10	0	No	
6		DI	3	20	10	0	No	
7		DI	3	20	10	0	No	
8		DI	3	20	10	0	No	
9		DI	3	20	10	0	No	
10	Load Sample	Load Until Empty (mL)	Syringe Fill Pause (s)	Sample Sip Rate (mL/min)	Sample Deliver Rate (mL/min)	Minimum Volume (mL)	Expected Volume (mL)	
		Yes	1	20	10	400	500	
11	N2 Purge Timer	Delay (min)						
		10						
12	Cartridge Elute	Reagent	Volume (mL)	Delivery Rate (mL/min)	Destination (Tube)	Soak (s)	N2 Purge (s)	Liquid Sense
		DCM	3	10	1	20	0	No
13	Cartridge Elute	Reagent	Volume (mL)	Delivery Rate (mL/min)	Destination (Tube)	Soak (s)	N2 Purge (s)	Liquid Sense
		DCM	3	10	1	20	0	No
14	Cartridge Elute	Reagent	Volume (mL)	Delivery Rate (mL/min)	Destination (Tube)	Soak (s)	N2 Purge (s)	Liquid Sense
		DCM	3	10	1	20	20	No

522: Initial Demonstration of Capability (IDC)

Restek
- ResPrep 6 mL, 2 g Coconut Charcoal Cartridges
- Rxi®-5Sil MS; 30 m x 0.25 mm x 0.25 µm

	Blank	LCS 1	LCS 2	LCS 3	LCS 4	LCS 5	LCS 6	LCS 7	Average	St Dev	RSD
Recovery as ug/L											
	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(ug/L)	(%)
1,4-Dioxane-d8	8.64	10.81	9.22	9.83	10.39	10.15	9.73	9.49	9.94	0.54	5.47
1,4-Dioxane	ND	3.17	2.60	2.98	3.13	2.94	3.00	2.71	2.93	0.21	7.13
Recovery as %											
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
1,4-Dioxane-d8	86.44	108.06	92.16	98.32	103.86	101.48	97.34	94.86	99.44	5.44	5.47
1,4-Dioxane	ND	105.53	86.53	99.40	104.27	98.07	100.00	90.33	97.73	6.97	7.13

Method 522 results were well within the ≤ 20% RSD criteria for the IDP. 1,4-Dioxane resulted in a %RSD of 7%. The IDA for 522 fell within the limits as well, which are established at ± 20% of the true value. The recoveries averaged 98% for 1,4-Dioxane.

Smart Prep Features and Benefits

Features	Benefits
Bottle Rinse (optional)	Less operator labor and better precision
Small Footprint	Preserves bench space and fits in most lab enclosures
Processing time is identical for each sample	Improved precision sample to sample
Pre-written Library of EPA Methods	Easy start-up right out of the box
Unattended operation	Accomodates lab workflow
Onboard Intelligence for Smart Sample prep	
Liquid Sensor	Volume verifiyer, for sample and solvent
	Auto calculator confirms sample volume used
	Reports generated at end of batch
Reagent Management Assitant	
Live solvent volume status reporting	Enables solvent volume levels to be monitored easily
Solvent volume calculator	Calculates how much solvent will be consumed & prompts
	the operator to ensure that enough solvent is available
	prior to the start of each run..
Modular & Scalable	System expands easily along with your sample demands
Multiple different methods can be performed on different	unprecedented flexibility to accommodate ever changing
Intuitive, Windows based PC software	Supports complex method development
Compatible with Acetone	Enables more methods and method development
8-reagents (solvents) supported	Broadest range of system solvents possible
Solvent Mixing Chamber	Flexibility to design methods around optimal chemistry
Inline membrane filter	Protects the entire system
Solid Sip Tube	Allows for total sample recovery from vessel.
"Chemistry-first" design approach	Insures that proper chemistry techniques are always
	applied- resulting in improved performance w/less effort

Results

- Automating your SPE process with the SmartPrep will allow you to achieve a higher level of consistent recoveries by controlling all critical extraction times and parameters. With the advances incorporated into the SmartPrep system many types of aqueous matrices will be extracted faster while improving data consistency.
- The SmartPrep has proven to be a convenient way to automatically calculate the liquid volume of the sample being processed consistently every time .This automated feature eliminates the need for the operator to mark and refill the bottle, and transfer this to a graduated cylinder which is very subjective and can result in final concentration errors.
- The SmartPrep's ability to automatically control the eluting volume, and automatically switch to another collection tube, will improve the accuracy and consistency of any method which requires, or desires, multiple fraction collections.
- The SmartPrep demonstrated its ability to rinse the sample bottle effectively. If the sample bottle walls are not rinsed effectively with the eluting reagent, many of the analytes would not be recovered. Many of the analytes of interest do adhere to the container walls resulting in low and inconsistent recovery values which were demonstrated with a manual rinsing technique.
- The SmartPrep Extractor System is a very unique and powerful instrument that can enhance all SPE cartridge operations currently being done manually, freeing up valuable operator time.

Horizon technology

Thank You

Questions?