

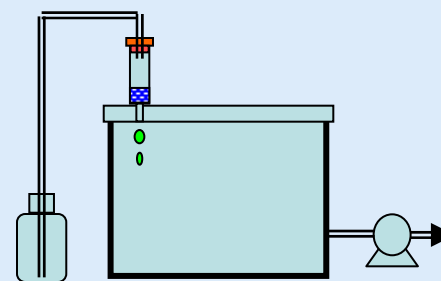
Automation of Solid Phase Extraction and Column Chromatographic Cleanup

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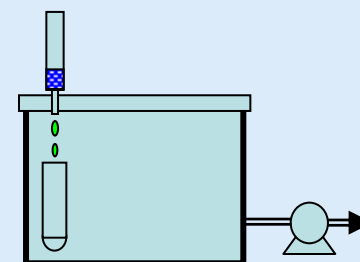
PC Tech.

Problems with Manual Operation

1. Unstable flow rate affects reproducibility and throughput.
2. Not convenient for multi step elution.
3. Labor intensive.



SPE of water sample



Elution of trapped analytes. Vacuum may be applied depending on the flow of solvent.

Advantages of Automated operation

1. Reduce exposure of operators to hazardous solvents.
2. Release chemists and technicians from tedious and labor intensive work.
3. Improve reproducibility and avoid human error.
4. Enable multiple step elution for complex sample cleanup.

Approaches for Automation

Off line approach

Automate the procedures in manual operations

On line approach

Integrate SPE with instrumental determination

SPE-01 and SPE-01 plus Cleanup Station

For offline automation of SPE and column chromatography

Designed for pesticides and drug residues in food samples

Computer-free

Up to 5 elution solvents

Collect 2 fractions for each sample

Difference:

SPE-01 Process 6 samples per batch, SPE01 plus process 9 samples per batch; SPE-01 plus use touch screen; Price for SPE-01 is 20% lower.



Computer free and easy operation

Step 1, edit method
(only necessary for
new method)



The screenshot shows a menu with tabs for 'Main', 'Config', 'Mthd 1', 'Mthd 2', and 'Mthd 3'. Below the tabs is a table with columns for 'Action', 'Flow', and 'Vol'. The table contains five rows of data.

| | Action | Flow | Vol |
|---|--------------|------|-----|
| 1 | Add Sample | 20 | 45 |
| 2 | Collect to 2 | 15 | 1 |
| 3 | Elute with 2 | 14 | 2.5 |
| 4 | Elute with 3 | 18 | 2.5 |
| 5 | Elute with 4 | 16 | 2.5 |

Step 2, pick samples
and method



Step 3, press start
button

An example of method for SPE-01 Plus

| n | Action | Flow rate | Volume |
|---|--------------|-----------|--------|
| 0 | Elute with 1 | 16 | 10 |
| 1 | Elute with 2 | 10 | 10 |
| 2 | Add sample | 4 | 5 |
| 3 | Elute with 2 | 5 | 5 |
| 4 | Elute with 3 | 5 | 1 |
| 5 | Collect to 1 | 5 | 25 |

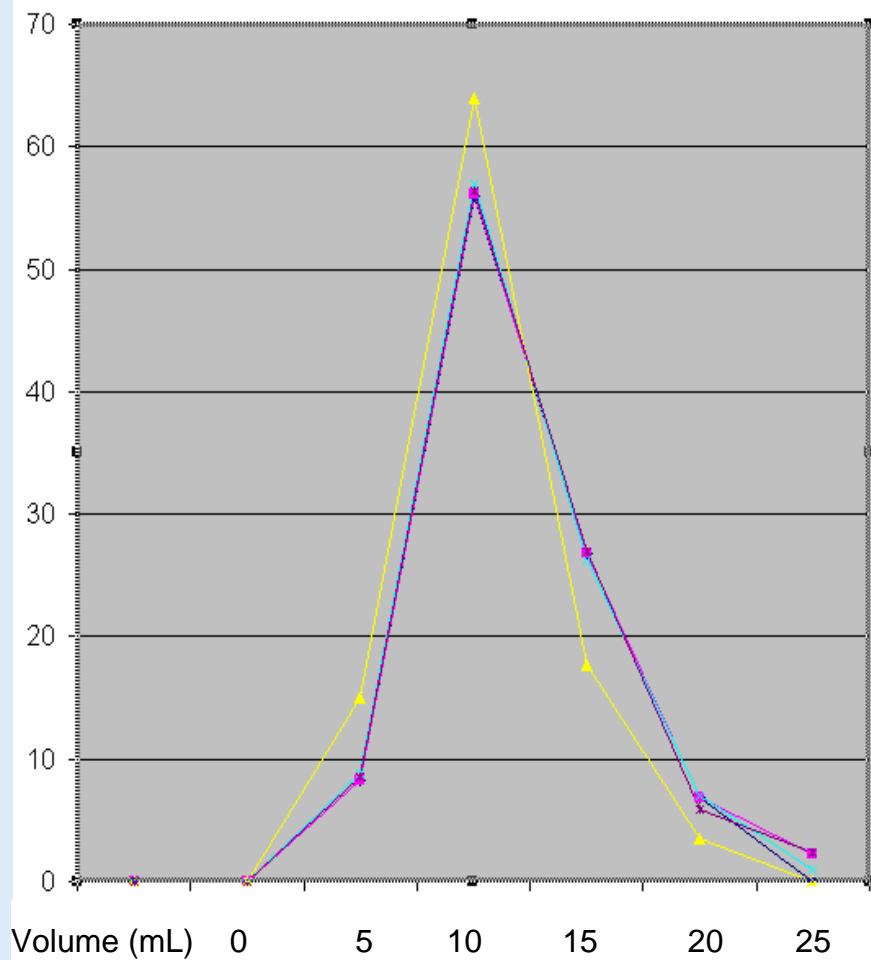
Eluent 1, acetone; eluent 2, hexane; eluent 3, hexane +5% acetone. Column, Florisil.

This method is for analysis of pyrethroid pesticides in tea. The Florisil column is first conditioned with acetone and followed with hexane. The concentrated extract is added to the column. It is then first eluted with hexane to remove interference. The targeted components are eluted using solvent 3 and collected for GC-ECD analysis.

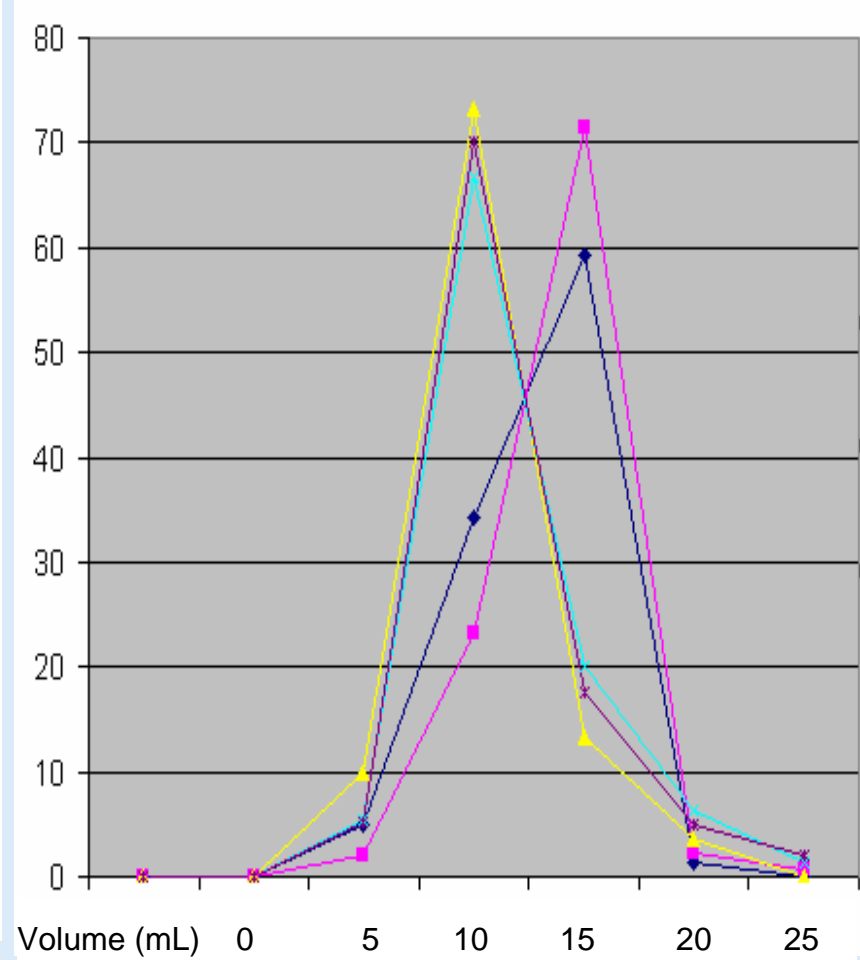
Elution pattern of 5 pyrethroid insecticides on column packed with 3 gram flowrisil (fenpropathrin, cyhalothrin, cypermethrin, fenvalerate, deltamethrin)

Data provided by Tea Research Institute, Chinese Academy of Agric. Sci.

Flow rate=6 mL/min



Flow rate=18 mL/min



Elution pattern of 5 pyrethroid insecticides on column packed with 3 gram flowrisil (fenpropathrin, cyhalothrin, cypermethrin, fenvalerate, deltamethrin)

(Continued)

- As shown in previous slide, there is no significant change in elution pattern when flow rate is increased from 6 mL/min to 18 mL/min. Much faster elution can be achieved using the automated SPE.

Comparison with other automated SPE

- SPE-01 and SPE-01 plus is specially designed for analysis of pesticide and drug residues in food samples. Most other automated SPE instruments are designed for clinical samples. The two applications have different requirements in sample volume, elution steps, and fraction handling.
- SPE-01 and SPE-01 plus use valves for liquid handling. Other automated SPE instruments use XYZ motion control. SPE-01 plus has lower cost and better reliability.
- The valve design enables SPE-01 plus to use different type of SPE columns, such as self packed florisil column, whereas other automated SPE need to use expensive adapters and can only handle commonly used dimension of SPE.
- SPE-01 plus does not need computer. Its small size saves the expensive lab space and makes it easy to be placed in a fume hood.

SPE-03 4-Channel SPE System for Water Samples

- Process 4 samples simultaneously
- Separated line for water and organic waste
- Blow dry function
- Detection and smart handling of blockage
- Computer free
- Small foot print

The major difference with SPE-01 plus is in number of pumps. SPE-03 has four pumps and SPE-01 plus has only one.



A comparison between manual and automated SPE

Tap water spiked with 9 triazole pesticides (三唑类农药) at 1 ppb level was extracted using both vacuum manifold and SPE-03.

| Pesticide | Recovery (%) | | Standard deviation (%) | |
|---------------|--------------|-----------|------------------------|-----------|
| | Manual | Automated | Manual | Automated |
| Triadimefon | 76 | 99 | 8 | 2 |
| Paclobutrazol | 83 | 97 | 14 | 6 |
| Hexaconazole | 88 | 100 | 14 | 5 |
| Uniconazole | 83 | 93 | 13 | 5 |
| Myclobutanil | 77 | 95 | 10 | 5 |
| Flusilazole | 90 | 93 | 5 | 6 |
| Propiconazole | 83 | 101 | 15 | 7 |
| Tebuconazole | 82 | 93 | 6 | 4 |

Data provided by Xiamen Powertech Technologies Ltd.

Online SPE - Integrate SPE with HPLC Analysis

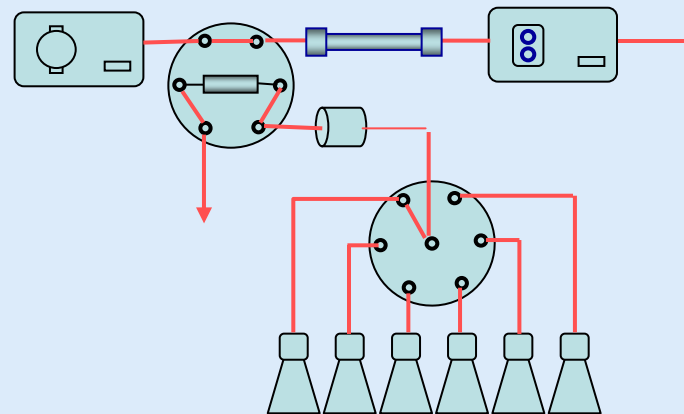
Two approaches:

1. One sample per SPE column approach.

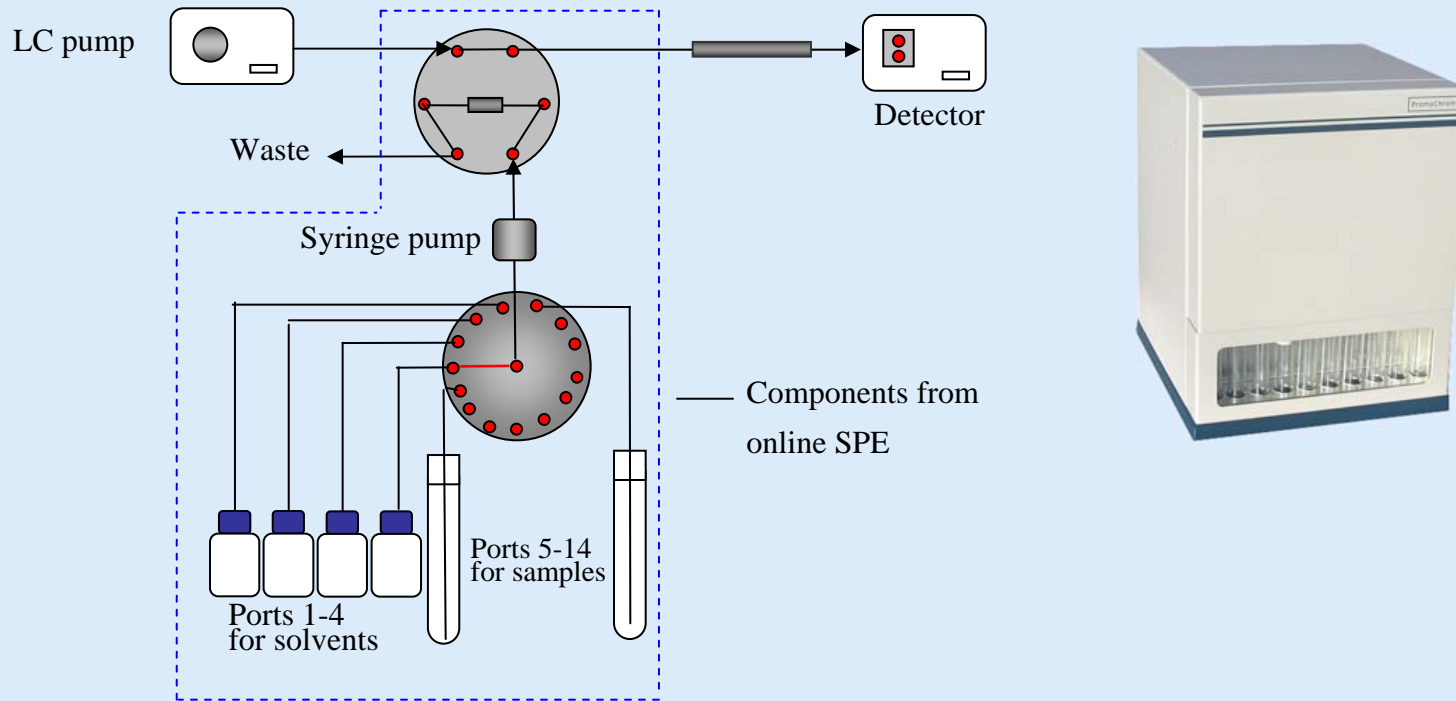
Easy to develop robust method. High cost.

2. Single SPE column approach

One SPE column for multiple samples. Much less expensive. Suitable for clean samples. For dirty samples, finding reusable SPE column is the key for success application.



Online SPE from PromoChrom



The online SPE looks after sample injection and elution of SPE column. The HPLC does not need a secondary pump and sampler. This is the online SPE system with the lowest cost.

Online SPE Control Software

- Real time display of flow path and instrument status;
- Overlapped injection to save time in sample loading
- Step run function for easy method development;
- Use method and sequence for analysis, similar concept as Agilent Chemstation;
- Easy integration with any HPLC;
- Diagnosis tool for trouble shooting.

The screenshot shows the 'Online SPE PromoChrom' software interface. At the top, there are menu options: Initialize, Configure, Report, Method, Sequence, Step run, and Diagnosis. Below the menu are 'Start' and 'Pause' buttons. The 'Run status' is 'Ready', 'Loaded method' is 'Med start.txt', and 'Time elapsed' is blank.

The central part of the interface displays a schematic diagram of the HPLC/SPE system, including two sample vials (A and B), a 10-position injection tray, a waste container (W), and various valves and pumps.

Below the diagram are two tables:

| Action | Parameter 1 | Parameter 2 |
|------------------|-------------|-------------|
| elute with 2 | 2.0 | 10 |
| elute with 1 | 2.0 | 5 |
| load sample | 3.0 | 5 |
| inject | 0.0 | 5 |
| start LC | 0.0 | 1 |
| wait | 0.1 | 5 |
| elute with 2 | 2.0 | 5 |
| elute with 1 | 2.0 | 5 |
| load next sample | 3.0 | 5 |

| Time | Vial | Method | Description |
|------|------|----------------|-------------------------|
| 0.00 | 1 | Med start.txt | 1 ppb caffeine standard |
| 1.50 | 3 | Med middle.txt | 10 ppb caffeine. |
| 3.00 | 2 | Med middle.txt | water ex Richmond |
| 5.00 | 4 | Medend.txt | Water ex Vancouver. |

Below the tables are control buttons: 'Amend', 'Insert', 'Edit', and 'Delete'. There are also dropdown menus for 'Method' (default.txt, Med middle.txt, Med start.txt) and 'Sequence' (default.txt, Overlay.txt, short.txt). A 'Save report' checkbox is checked, and a 'Report name' field is present.

Direct Analysis of PAH in Tap Water at ppt level

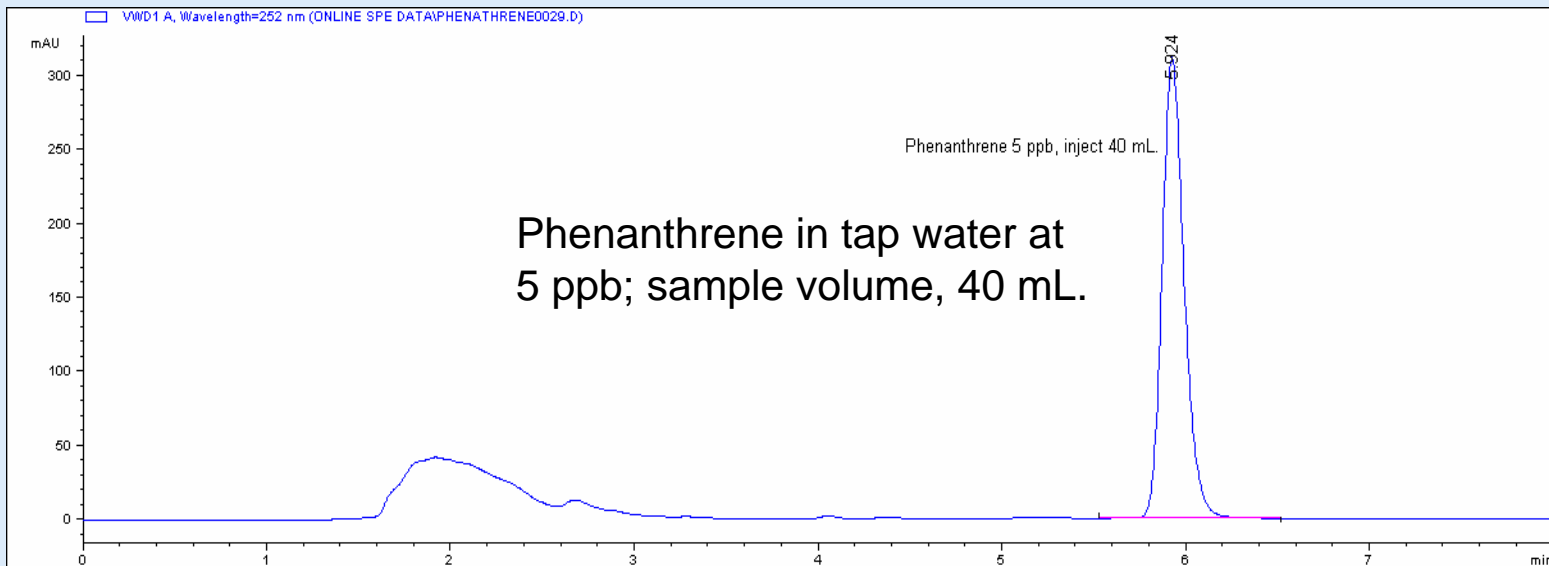
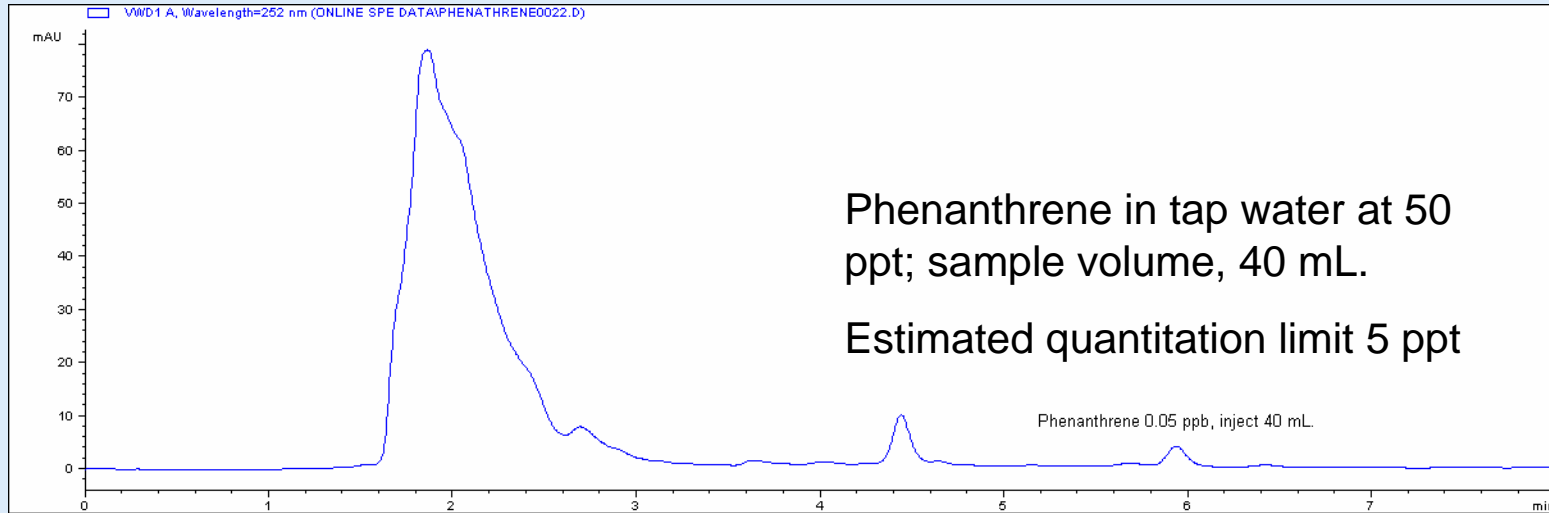
HPLC conditions:

Mobile phase, methanol + water (90:10);
Flow rate, 1.5 mL/min;
Column, PromSil C18 4.6x250 mm;
Detection, 252 nm, Agilent G1314A VWD ;
Stop time, 8 minutes.

Online SPE procedures:

Elute with 2 mL methanol at 6 mL/min;
Elute with 3 mL water + methanol (95:5,) at 6 mL/min;
Load up to 40 mL sample;
Inject sample to HPLC column;
Start HPLC analysis.
SPE column, Trap N, 4.6 x 10 mm.

Direct Analysis of PAH in Tap Water at ppt level



Direct Analysis of PAH in Tap Water at ppt level

Repeatability (peak area mAU)

| Repeat | 5 ppb (20 mL) | 0.1 ppb (20 mL)* | 0.01 ppb (40 mL)* |
|---------|---------------|------------------|-------------------|
| 1 | 1273 | 39.9 | 26.5 |
| 2 | 1249 | 37.0 | 26.0 |
| 3 | 1239 | 38.4 | 27.3 |
| Average | 1254 | 38.4 | 26.6 |
| CV% | 1.4 | 3.8 | 2.5 |

*As the tap water has 0.04 ppb phenanthrene, the 0.01 level should be 0.05 ppb.

Comparison Between Offline and Online Approach

| Items | Offline SPE | Online SPE |
|----------------------|---|---|
| Analysis time | Over 1 hours per sample. This includes extraction of 1 liter water sample, concentrate to 1 mL, and use 10-20 uL for HPLC analysis (equivalent to 10-20 mL water sample). Over 90% of processed sample is not used. | 15 minutes per sample. All of 20-40 mL sample is injected to HPLC column. The SPE procedures are carried out while the HPLC is analyzing previous sample. |
| Material cost | One SPE column per sample (US\$1.5-2), 20-30 mL methanol for conditioning and elution. | One SPE column for 50-100 samples, US\$0.5-1 for 1 sample; 2 mL methanol for each sample. |
| Data quality | RSD above 5% at sub ppb level | RSD <5% even at 50 ppt. This is due to simplified and automated procedures. |
| Suitable application | Semi volatile pollutants in water, in which determination is by GC or GC-MS | Chemicals that can be analyzed by HPLC or LC MS |