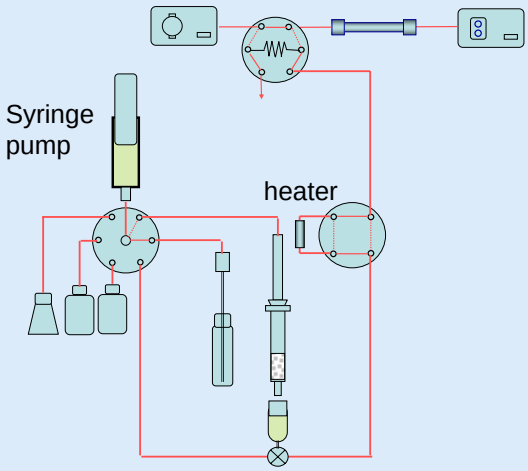
Integration of SPE with LC and LC-MS analysis

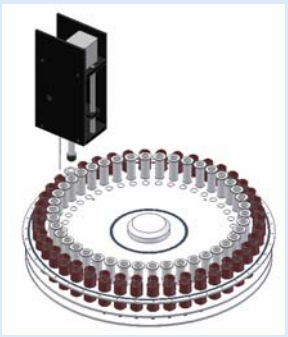
PromoChrom Technologies Ltd.

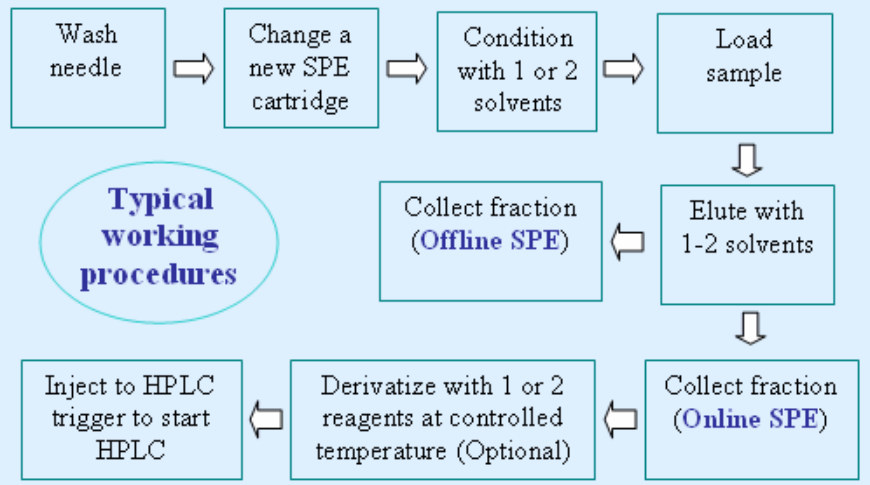
Integration of sample cleanup with instrumental analysis helps to improve data reproducibility and increase sample throughput. It can also release chemists from labor intensive routines. PromoChrom developed two solutions for the integration.

Solution 1: SPE-04 multi functional SPE

The instrument can perform both online and offline SPE. In addition, it has a function for online derivatization at controlled temperature after column cleanup (between column derivatization).







Application example: Analysis of hormones in plasma sample

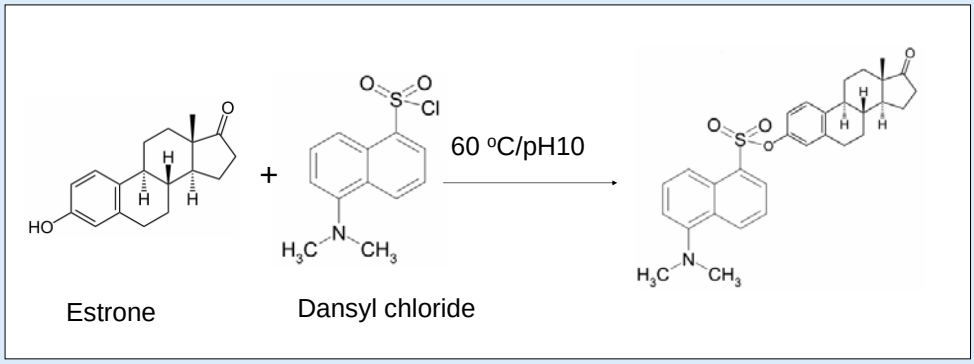
1.Remove protein from plasma by addition of acetonitrile and centrifuge

2.Take 0.5 mL sample and dilute to 4 mL using water

3.Load sample to a HP SPE C18 column (50 mg/3 mL) and wash column with 2 mLwater.

4.Elute with 0.5 mL methanol and derivatize the fraction with a basic buffer and dansyl chloride.

5.Inject into HPLC and start analysis using a C18 column for separation and UV detection at 360 nm.



Features of SPE-04

1. Offline SPE

It can work for offline column cleanup and fraction collection. When working in offline mode, computer is not necessary. Users can set up the instrument quickly using the 7 buttons. Easy operation. The offline mode does not need computer.

2. Online SPE

In online mode, the collected fraction is directly injected into an HPLC or LC-MS for final determination. The control software for online SPE is user friendly and is compatible with most HPLC software.

3. Direct sample injection

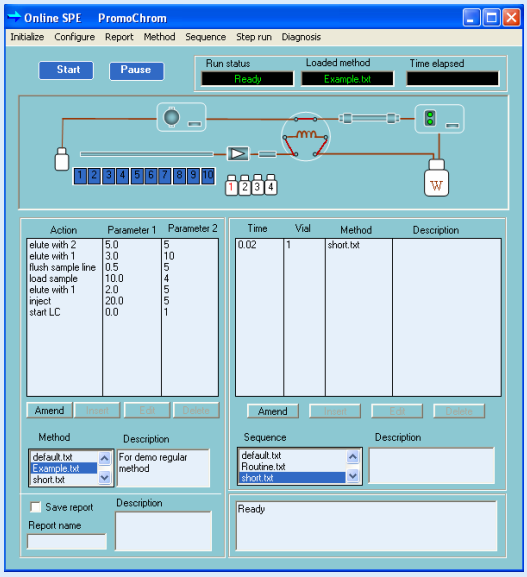
SPE-04 can work as an auto sampler. Therefore, the HPLC does not need to have another sample introduction device.

4. Derivatization after cleanup and at controlled temperature

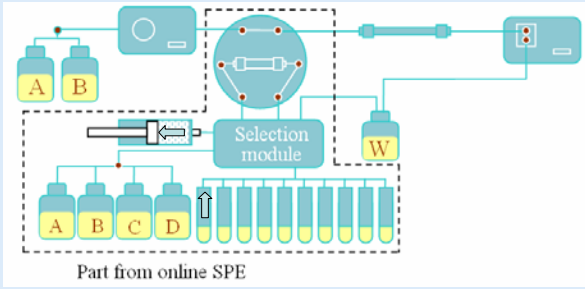
Conventional pre column derivatization is normally carried out at ambient temperature. The reaction temperature limits the selection of derivatization reagents. Besides, the sample matrix may interfere the reaction if a cleanup has not been performed. By providing cleanup before derivatization and a well controlled temperature, SPE-04 gives more reliable derivatization results.

Solution 2: LC-03 online SPE for Direct Analysis of Liquid Samples

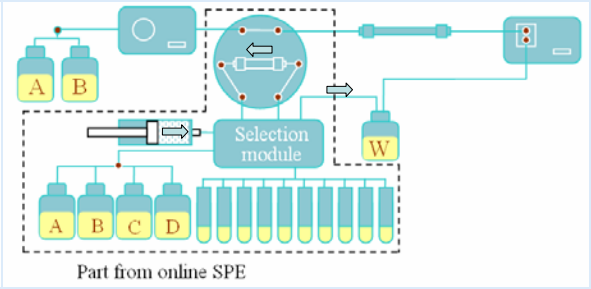
LC-03 online SPE uses valves and a syringe pump to transfer sample to SPE column and then to the HPLC system. The control software looks after the detailed valve switches. Users only need to select the necessary actions. Such as “load sample”, “add reagent 1”, “inject”, “start LC”, etc.



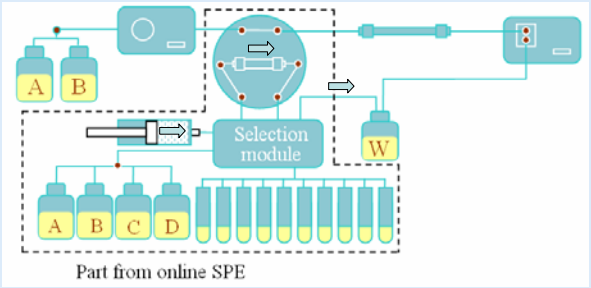
Working procedures of LC-03:



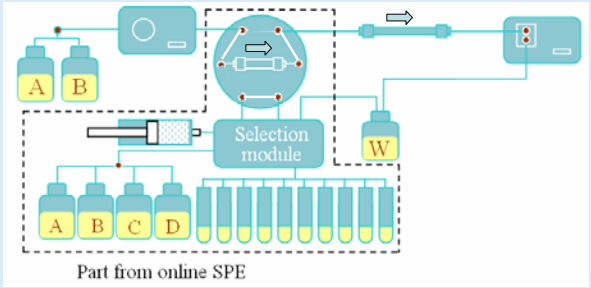
1. Draw sample or elution solvent



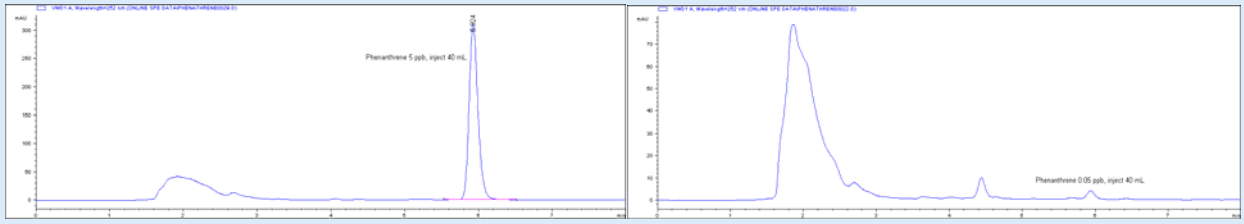
2. Load sample to SPE column in reversed direction



3. Wash SPE column in normal direction to remove interference and small particles



4. Elute trapped analytes to HPLC column



Direct analysis of phenanthrene in tap water at 5 ppb and 50 ppt level using online SPE coupled with HPLC. SPE column, TrapN; analytical column PromSil C18; sample volume, 40 mL; flow rate for sample loading, 6 mL/min; detection wavelength, 252 nm.

Features of LC-03

1. Allow elution of SPE column in two directions

It helps to prevent trapped particle from entering analytical column and obtain a narrow band when eluting the trapped analytes from SPE column to HPLC column.

2. Standalone operation

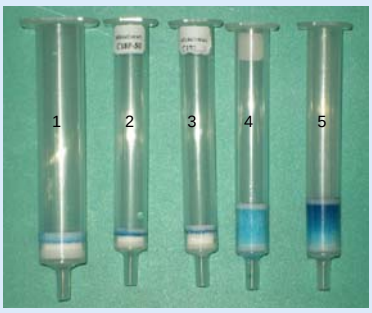
The device can be controlled using built-in methods for most applications. It is easy to hook up to any LC or LC-MS system without disturbing the computer environment.

Comparison of the two online SPE solutions:

	SPE-04	LC-03
Use of SPE column	One SPE column for each sample. More tolerant to dirty samples. Wider selection of suitable SPE columns.	One SPE column for multiple samples(50-100). Less selection of SPE columns due to mobile phase compatibility issue. More suitable for clean samples.
Use of sample	Trapped analytes are washed to a vial and then loaded to injection loop. Only a portion is used for analysis	Trapped analytes are directly introduced to HPLC column. 100% of trapped analytes are analyzed.
Number of samples	Up to 41 samples per batch	Up to 10 samples per batch
Volume of samples	Up to 20 mL	Up to 100 mL

Use high performance SPE column to improve sensitivity Of SPE-04

The HP SPE column is based on high quality spherical silica gel and more complete bonding process. Below is a comparison using a blue dye aqueous solution:



1. 100mg/6mL HP SPE C18
2. 50mg/3mL HP SPE C18
3. 100mg/3mL HP SPE C18
4. 500mg/3mL conventional SPE C18
5. 500mg/3mL conventional SPE C18

Trapped dye can be eluted out using less than 0.5 mL acetone

As SPE-04 can only make use a portion of the trapped analytes for analysis, enrichment by SPE column is important for improving detection sensitivity. By using a 50-mg HP SPE column, a 5-mL sample will end up in 1 mL fraction. It is enriched 5 times.