PROMOCHROM TECHNOLOGIES

Online SPE

SPE-04

Direct integration of solid phase extraction with LC/LC-MS to achieve higher levels of automation, efficiency and sensitivity

About PromoChrom

PromoChrom Technologies focuses on the development of sample preparation solutions for trace analysis.

Since **2005**, PromoChrom has developed the SPE-01 and SPE-03 cleanup stations, SPE-04 online/offline SPE's, LC-04 online SPE, RT-01 sample purifier and SPE-06 mini SPE. Each of the instruments target specific applications.

In **2011**, PromoChrom developed the flow-path-integration technique for liquid handling. It combines various switching valves into one liquid handling module. This technique simplified the structure of our instruments considerably, making the instruments more affordable and more reliable.

In **2017**, "Two-tier online SPE" was invented by PromoChrom which uses a second SPE column for online SPE. This method significantly increased the detection sensitivity and mitigated column compatibility and clogging issues commonly found in online SPE systems

Today, we continue to look for more opportunities and breakthroughs in laboratory process automation

SPE-04

The next step.

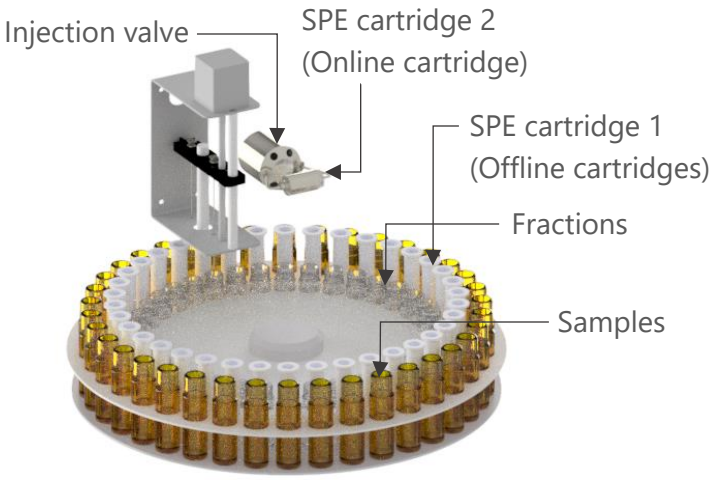
SPE-04 multi-functional solid phase extraction system is a flexible and versatile sample preparation platform that can be paired with any LC/LC-MS system with a remote port. It is designed for automatic cleanup of biological (eg. small molecules in plasma/urine), food and environmental samples. This online SPE system is fast and uses much less sample and organic solvents. Compared with offline SPE, it can achieve similar detection limits with just 1-2% of the sample volume since all the analytes can be transferred to the analytical column. SPE-04 can perform offline/online solid phase extraction, normal sample injection, online derivatization with controlled temperature and our proprietary two-tier online SPE. The two-tier approach provides better sensitivity, longer SPE cartridge life and wider application range than typical online SPEs.

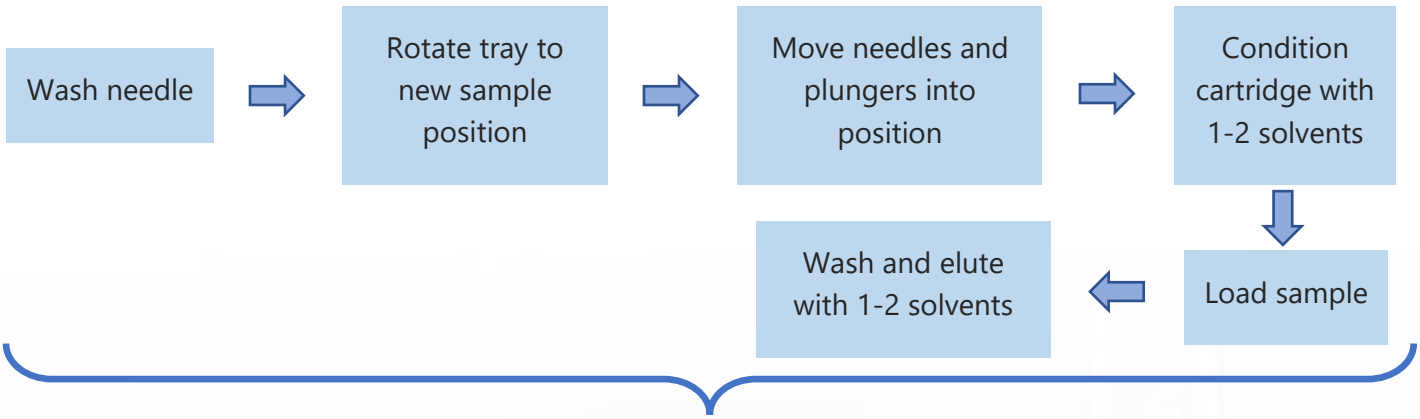
1. Working Principle

Simple and robust design

Up to 36 sample vials, SPE cartridges and fraction bottles can be loaded onto the circular tray. The needle head controls 2 needles for samples and fractions and a plunger that forms a seal around the offline SPE cartridges to deliver fluid using positive pressure. A sample loop or online SPE cartridge is connected to the injection valve depending on the configuration (indirect vs direct/two-tier online SPE)

The following diagram describes the typical working procedures of SPE-04.





Offline SPE

Collect fraction

Online SPE (indirect)

Derivatize with 1 or 2 reagents at controlled temperature (optional)

Load fraction onto sample loop

Trigger HPLC start

Two-tier

Derivatization and/or solvent modulation

Load fraction onto 2nd SPE cartridge

Remove particles and interferences

Trigger HPLC start

2. Features

Versatile and Easy to Use

Multi-functional Platform

Depending on the configuration, the SPE-04 can perform offline SPE, online SPE (indirect, direct or two-tier) and online derivatization. The online derivatization function is very useful for the analysis of amino acids, hormones and certain pesticides

Online Derivatization

Online derivatization can be performed on the collected fraction with 1-2 solvents and controlled temperatures of up to 80 deg C

Indirect Online Injection

Using a built-in injection valve, the SPE-04 can load the collected fraction onto the sample loop which is then delivered to the HPLC column using the HPLC pump

Direct and Two-tier online SPE

Direct online SPE replaces the sample loop with an online SPE cartridge, allowing more analytes to be trapped and transferred to the HPLC column. When used in conjunction with the offline SPE cartridges, it achieves two-tier SPE which combines the advantages and eliminates the challenges of direct and indirect online SPE. All of the analytes can be transferred to the HPLC column to give high sensitivity and excellent peak shape. Solvent modulation after fraction collection takes care of incompatibility issues between the SPE and HPLC columns. By using an innovative cleaning method, the 2nd SPE cartridge can be regenerated over 100 samples. Learn more at : https://www.promochrom.com/applications.html

Different Sample container sizes

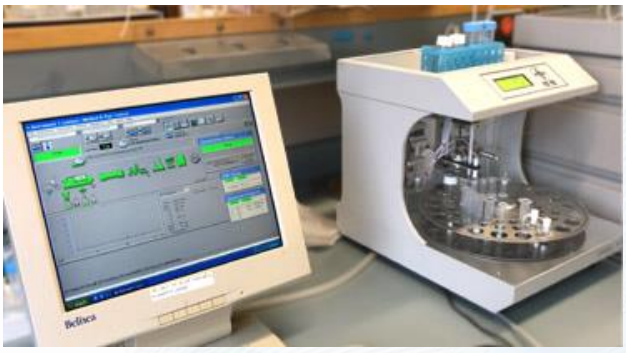
The SPE-04 is compatible with 4mL, 8mL or 20mL sample vials using different tray configurations

Different SPE Cartridge sizes

The SPE-04 is compatible with 1mL, 3mL or 6mL SPE cartridges using different tray configurations

Parallel Integration with LC/LC-MS

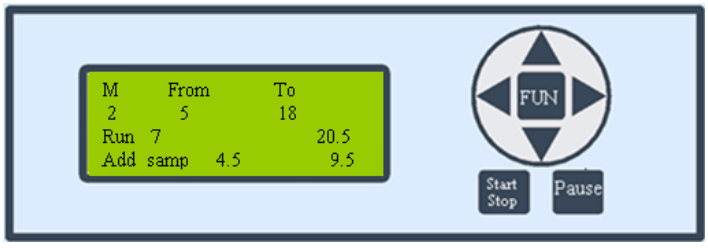
The SPE-04 can be coupled with any LC or LC-MS system that has a remote trigger port. There is no need to alter the LC/LC-MS software. Once the sample is cleaned up and loaded onto the injection valve, the SPE-04 signals the LC/LC-MS to begin analysis and immediately starts processing the next sample. This significantly reduces the time and handling steps required for analysis



Easy Operation

SPE-04 can store up to 3 methods without the need of an external computer. Operation of the instrument involves only 7 buttons. Below is a typical routine operation procedure:

- Place samples and SPE cartridges on the tray
- Select samples to be processed
- Select method
- Press the start/stop button



The screen indicates that samples 5 to 18 are to be processed using method 2. It is now performing the add sample action on sample 7. The sample intake is set to 20.5 mL for each sample.

When using online SPE mode, the collected fraction is injected into an HPLC or LC-MS for final determination. The control software for online SPE is user-friendly and does not have to interface with the HPLC software. Using a similar structure as Agilent Chemstation (methods and sequences), users familiar with HPLC can easily understand the SPE-04 software.

When the HPLC is performing analysis, SPE-04 can start processing the next sample, thereby increasing throughput from overlapped injection

3. Accessories

Catered to Your Application

MOD-001

Two-tier online SPE

A 2nd specialized online SPE cartridge replaces the sample loop on the switching valve. This eliminates bottlenecks in conventional online SPE such as incompatibility between SPE and HPLC columns or column clogging. Other benefits include:

- Reduced peak broadening
- Reduced background noise
- Significant detection limit improvement through large volume sample introduction

MOD-002

Online Derivatization

A controlled heater block is added to allow derivatization of fraction before loading it onto the sample loop or online SPE cartridge on the injection valve

Rotating Tray

Part No.	Sample Vol.	Fraction Vol.	SPE Cartridge	Samples
S-04-R-20-3 (default)	20mL	8mL	3mL	25
S-04-R-20-6	20mL	8mL	6mL	19
S-04-R-08-3	8mL	4mL	3mL	36
S-04-R-08-1	8mL	4mL	1mL	36

* The maximum volume for samples and fractions of a tray can be swapped by exchanging the tubing connection of the sampling needles. For example, tray S04R-08-3 can have 4mL sample volume and 8mL the fraction volume instead.

SPE Cartridges

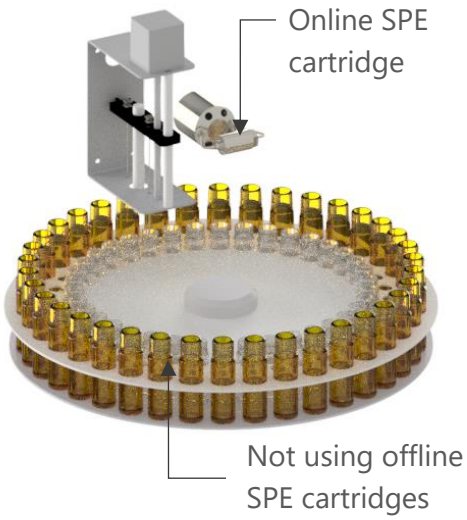
We provide a wide selection of 1mL/3mL/6mL C18, PSA, SCX, SAX and PEP cartridges. For more information on part number and pricing, please visit:

https://www.promochrom.com/columns.html

4. Application Example

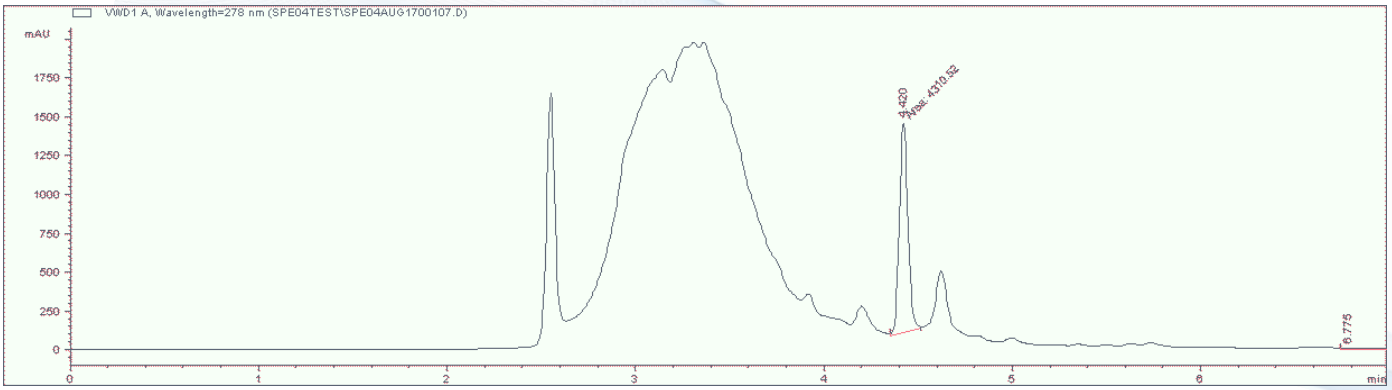
Analysis of chloramphenicol in honey using direct online SPE

Using just the online SPE cartridge (Trap N, C18), a 5 mL water solution containing 1.0 gram of honey and 4 ug chloramphenicol was loaded. The online SPE cartridge was washed using a special method to remove the interferences and particles. The trapped sample was then transferred to HPLC. The elution program B was used for HPLC separation with a PCTsil C18 (4.6X200mm) column. Below is a chromatogram for the spiked sample.



Program B

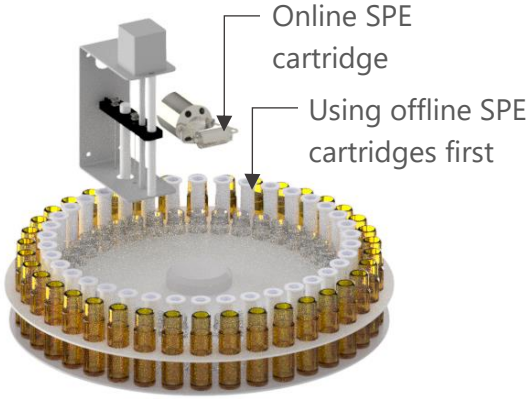
Acetonitrile and water as mobile phase. Increase acetonitrile from 10 to 70% over 3 minutes, hold for another 3 minutes, then return to 10% within 1 minute.



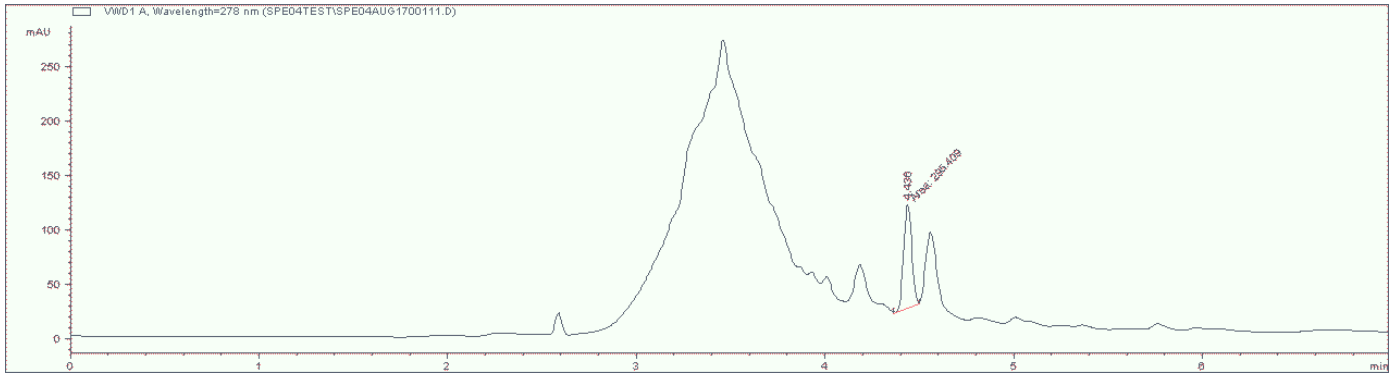
The estimated detection limit based on the peak height is 0.2 ppm. Processing time for one sample is 10 minutes. After 20 samples, the SPE cartridge began to show blockage. The following experiment demonstrates how the two-tier approach can extend SPE cartridge lifetime and improve sensitivity.

Analysis of chloramphenicol in honey using two-tier online SPE

In this experiment, the sample was first cleaned using a C18 cartridge from PromoChrom (200 mg/3-mL) before loading the fraction onto the online SPE cartridge. Subsequent steps were unchanged. Detection was possible even at much lower sample concentrations (4 mL water solution containing 1.0 gram honey and 0.5ug chloramphenicol).



Below is a chromatogram of the sample.



In comparison with the chromatogram using direct online SPE, the two-tier approach has reduced the background considerably. The detection limit was also improved due to reduced background interference. It is estimated at 0.05 ppm. The lifetime of the second SPE cartridge also extended considerably. One SPE cartridge worked well with no blockage or loss of trapping capability through out the following experiments for honey and urine samples (over 60 samples). The processing time for one sample is 16 minutes (includes the time for HPLC analysis). Below is the detailed method for SPE-04 instrument:

Action	Flow rate	Volume	Remarks
Elute 1	5 mL/min	2.5 mL	Pre-condition the offline SPE cartridge using 2.5 mL methanol (solvent 1)
Add Samp	5 mL/min	4.0 mL	Add 4.0 mL sample to the offline SPE cartridge
Elute 2	5 mL/min	7.5 mL	Wash offline SPE cartridge with 7.5 mL water (solvent 2)
Elute 1	5 mL/min	0.1 mL	Change elution solvent to methanol (solvent 1)
Collect	5 mL/min	1.0 mL	Continue elution with methanol and collect 1 mL fraction
Mix Frac	2	3.0 mL	Mix 1.0 mL fraction with 3.0 mL water (solvent 2) in the mixing chamber
Load Mix	4 mL/min	1.5 mL	Load 1.5 mL mixed solution onto the online SPE cartridge
Wash 2	4 mL/min	0.5 mL	Wash the online SPE cartridge with 0.5 mL water (solvent 2)
Inject	1 mL/min	1.0 mL	Inject the trapped analytes into HPLC and trigger the start of LC analysis
Wait	1 mL/min	2.0 mL	Wait 2 minutes for all analytes to be transferred to LC

5. Specifications

Parameters	Details
Sample capacity	25 (20mL sample vial) or 36 (4mL/8mL sample vial) per batch
Volume of sample	4, 8 or 20mL
Material of wetted parts	Teflon, PEEK, 316 Stainless steel or ceramic, Pyrex glass
System control	LCD and keypad, Computer (online SPE only)
Solvent capacity	4 solvents (can request up to 6)
Flow rate	1 to 30 mL/min
Derivatization temperature	Ambient to 80 °C
Method functions	**Elute 1-4** – pre-condition or wash cartridge with selected solvent(s)
	Add Samp – load sample onto SPE cartridge
	Blow Air – Deliver air through SPE cartridge using syringe pump
	Collect – elute with selected solvent(s) and collect eluent in fraction tube
	Rinse 1 or 2 – Rinse sample container with and deliver rinsate through SPE cartridge
	Load samp/frac/mix – Load sample, fraction or derivatized fraction onto sample loop or online SPE cartridge
	Mix samp/frac – Add selected solvent into sample or fraction container and mix
	Wash/wash 1 or 2 – Wash sample loop or online SPE cartridge with solvent 1 or 2 in the forward or reverse direction
	Heat – Weat fraction for specified duration at set temperature
	Inject – deliver analytes from injection valve into HPLC
Pressure limit of pump	6 bar
Pump reproducibility (C.V. %)	<1.5 %
Power consumption	Input: 110-240Vac @ 25W, Output: <1A at 24 VDC
Weight	12 kg
Dimensions	34 cm x 42 cm x 35 cm (width x depth x height)

6. Ordering Info

Part No.	Description
SPE-04-01	Offline SPE only
SPE-04-02	Offline and online SPE (using sample loop)
MOD-001	Add 2nd tier SPE to SPE-04-02
MOD-002	Add derivatization to SPE-04-02
S-04-R-x-x	Rotating tray for different sample and SPE cartridge sizes. Available options are: S-04-R-20-3 S-04-R-20-6 S-04-R-8-3 S-04-R-8-1

PromoChrom Technologies

info@promochrom.com
1-833-772-4766
Unit 6, 12180 Horseshoe Way,
Richmond, BC V7A 4V5
Canada