TWO-TIER ONLINE SPE AND LARGE VOLUME INJECTION FOR LC AND LC-MS

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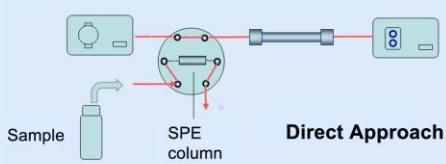
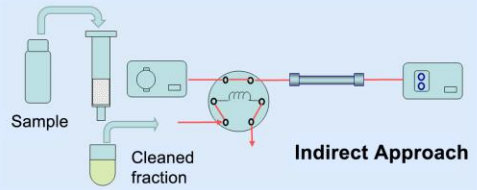
Integration of solid phase extraction or column cleanup with chromatographic analysis may take two approaches: 1) Direct coupling, in which a sample loaded in a cleanup column is completely transferred to the analytical column using a switching valve; 2) Indirect coupling, in which samples are treated like in conventional standalone SPE cleanup. A portion of the collected fraction is then injected into the analytical column using a built-in autosampler or by the sampler from the HPLC.





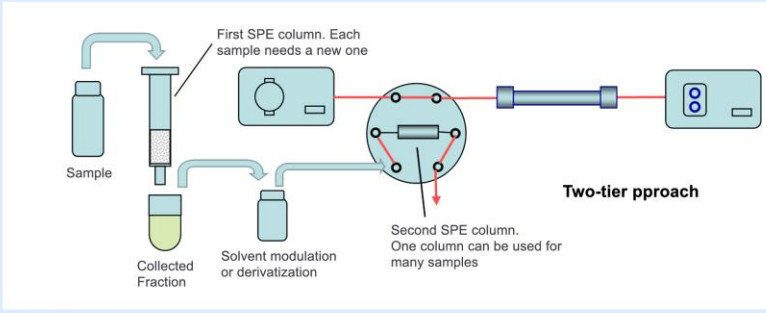
Using the direct approach can achieve higher sensitivity and faster sample throughput since all the treated sample is used for final analysis and the procedure is straightforward. For instance, in the analysis of trace pollutants in drinking water using the indirect approach, about 500mL of sample is normally used. The collected fraction is concentrated to 1 mL and an aliquot of 20 uL is injected for LC analysis. Essentially, only 10mL from the original 500 mL is used for final analysis, resulting in low sensitivity. If an online SPE is directly coupled with a LC or LC-MS, a 10 mL water sample can achieve the same sensitivity and the time for sample extraction is reduced from 2 hours to 10 minutes. However, the challenge with the direct approach is finding a suitable SPE column that is compatible with the analytical column and can be regenerated easily as it is difficult to change an online SPE column installed on a high pressure switching valve. The indirect approach avoids this problem as the SPE column is decoupled from the HPLC column and only a very small volume is injected into the HPLC.

Combining the benefits and eliminating the challenges

To overcome the disadvantages of the two online SPE approaches while maintaining their advantages, PromoChrom developed a 2-tier online SPE solution. Samples are first processed like in case of standalone SPE. The collected fraction is then modified using a solvent and then transferred to a second online SPE column on a switching valve. The first SPE treatment is used to remove most of the interference. The columns used are the same as those for standalone SPE packed with C18, ion exchange, or mixed mode sorbents. The second SPE column is used for enrichment and further cleanup. For example, salt from the first SPE treatment or the leftover reagents from the derivatization reaction can be removed by the second SPE column. Since the fraction entering the second SPE column has been cleaned by the first SPE column, the lifetime of the second column is considerably extended. The following diagram explains the working principle of this two-tier online SPE solution.

Instrumentation

SPE-04 online/offline SPE system is a flexible and versatile platform for automatic sample preparation. It can perform multiple tasks: offline SPE, indirect online SPE, direct online SPE, and 2-tier online SPE. It is also capable of normal sample injection, and online derivatization with controlled temperature. In the present application note, a SPE-04 system is coupled with an HPLC to perform the analysis of chloramphenicol in honey and tap water. The HPLC is an Agilent 1100 system with a binary pump and a UV detector.



Experimental

1. Analysis of chloramphenicol in honey

The chloramphenicol standard was from Sigma with a 98% purity. A 1 mg/mL chloramphenicol solution in methanol was prepared as stock solution. It was further diluted to 20 ug/mL and 2 ug/mL using methanol for spiking into samples and for HPLC analysis. The first SPE column was C18 200mg/3mL from PromChrom. The second SPE column was a Trap N (4.6X10mm) from PromoChrom. The HPLC columns were PromoChrom's PCTsil C18 columns (4.6X200mm, 5 um). Flow rate was set as 1.5 mL/min. The detection was at 278 nm. Acetonitrile and water were used as mobile phase. Gradient program: increase acetonitrile from 10 to 80% over 3 minutes, hold for another 3 minutes, then return to 10% within 1 minute. The honey used for experiment was named as "pure natural honey" by McCormick Canada.

2. Analysis of PAHs in river water

The PAH standard is a EPA 525 PAH Mix B from Supelco at 500 ug/mL in acetone. It was diluted to 12.5 ug/mL and 1 ug/mL using IPA for spiking into samples and for HPLC analysis. The first SPE column was C18 200mg/3mL from PromChrom. The second SPE column was a Trap N (4.6X10mm) from PromoChrom. The HPLC columns were a PromoChrom's PCTsil PAH column (4.6X250mm, 5 um). Flow rate was set as 1.2 mL/min. The detection was at 254 nm. Acetonitrile and water were used as mobile phase. Gradient program: increase acetonitrile from 40 to 80% over 10 minutes, increase to 100% at over 10 to 20 min. maintain till 44min and then go back to 40% and reduce flow rate to 0.3 ml/min at 45min.The water sample used for experiment was collected from Bear-Creek park in Surrey, British Columbia. The water in the river is mainly from rain fall.

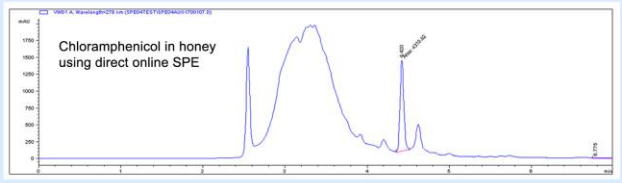
Results and Discussion

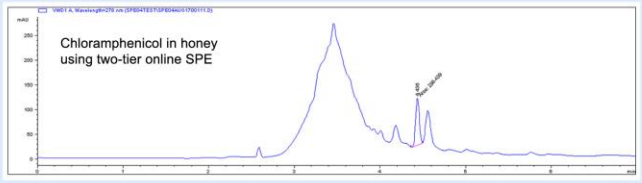
1. Analysis of chloramphenicol in honey using direct approach

A 5 mL water solution containing 1.0 gram of honey and 4 ug chloramphenicol was loaded to the second SPE column (Trap N). The SPE column was washed with 5 mL water in same direction as sample loading and then washed with 1.5 mL water in the reversed direction (to remove the particles). The trapped sample was then injected to HPLC. Below is a chromatogram for the spiked sample. The estimated detection limit based on the peak height is 0.2 ppm. The processing time for one sample is 10 minutes, including sample cleanup and analysis. After running 20 samples, the SPE column began to show blockage. For better sensitivity and longer lifetime of the SPE column, the wash procedures need to be optimized. A treatment using liquid/liquid partition or a offline SPE prior to the online SPE should also be helpful. The following experiment demonstrates the benefits of using the two-tier approach in extending the SPE column lifetime and improving sensitivity.

2. Analysis of chloramphenicol in honey using two-tier approach

After running 20 samples using the direct approach, the SPE column began to show blockage. This experiment demonstrates the benefits of using the two-tier approach in extending the lifetime of SPE column and improving the sensitivity samples of complex matrix. A 4 mL water solution containing 1.0 gram of honey and 0.5 ug chloramphenicol was first cleaned using a C18 column from PromoChrom (200 mg/3-mL). The column was washed using 7.5 mL 10% methanol. Then 1 mL fraction was collected using methanol as elution solvent. The fraction was mixed with 3 mL water (solvent modulation) and then loaded to the second SPE column. The second SPE column was then washed with 0.5 mL water in the direction opposite to sample loading to remove particles from the sample. The trapped analyte was then injected to the HPLC.

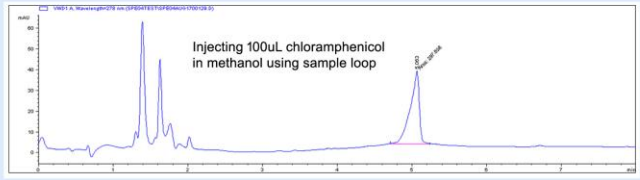


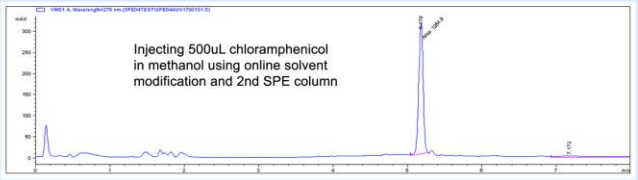


In comparison with the chromatogram using direct online SPE, the two-tier approach has reduced the background considerably. The detection limit improved due to reduced background interference. It is estimated as 0.05 ppm. The lifetime of the second SPE column also extended considerably. One SPE column 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 (including the time for HPLC analysis).

3. Large volume sample injection using SPE-04

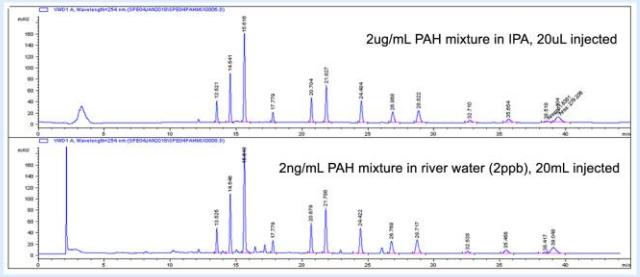
It is a normal practice in a HPLC analysis to make a sample's solution match the composition of the HPLC mobile phase. The injection volume for a 4.6-mm column is normally limited to 10-20 uL. If the sample solution and the LC mobile phase are not compatible or if the injection volume is too large, peak broadening will reduce the separation efficiency and the sensitivity. Using the function of online solvent modulation and the second SPE column in SPE-04 for enrichment, such adverse effects can be avoided. In the present experiment, a methanol solution containing 2 ug/mL chloramphenicol in methanol was introduced into the LC column using a 100 uL loop versus the second SPE column respectively. When using the loop, peak broadening was significant. For large volume injection using automatic solvent modulation and enrichment with the second SPE column, the injection volume of sample was increased to 500 uL. It was first mixed with 1.6 mL water and then loaded to the second SPE column and then injected into the HPLC. As shown in the chromatogram below, a 500 uL sample in methanol still gave better peak shape. In trace analysis, further concentration and solvent exchange are often necessary after sample cleanup using SPE or QUECHERS. The large volume introduction approach of SPE-04 can help to avoid these problems.





4. Analysis of PAHs in river water

River water was left to stand for 2 hours to allow visible particles to settle down. It was spiked with PAH standards at 2ng/mL (2ppb) level and used for analysis without filtration. Using direct approach, a 20-mL sample is added to the second online SPE column. The SPE column is then washed with 5 mL water before injecting into the HPLC. The direct approach of 20mL sample generated peaks as good as a 20 uL injection using sample loop. Although the water samples were not filtered, the SPE column had no blockage and the column head pressure of the HPLC column had no change after processing over 20 samples. This is because SPE-04 can wash the online SPE column in two directions for effective removal of particles and other interferring materials. The detection limit of the method is at ppt level even with a UV detector. It should be 10 to 20 times better when a fluorescence detector is used. The time for processing each sample is the same as the HPLC analysis time. The time of SPE treatment for each sample is 17 minutes and is performed in parallel with the HPLC analysis.



Phenathrene in tap water at 4 ppb level was used to evaluate the repeatability of this method based on direct online SPE. The figure on the right is the result of 5 analyses. Each analysis used 25 mL of sample.

	Run 1	Run 2	Run 3	Run 4	Run 5
Peak	8.679	8.675	8.697	8.675	8.677
Area	152.062	153.001	155.527	154.728	157.078