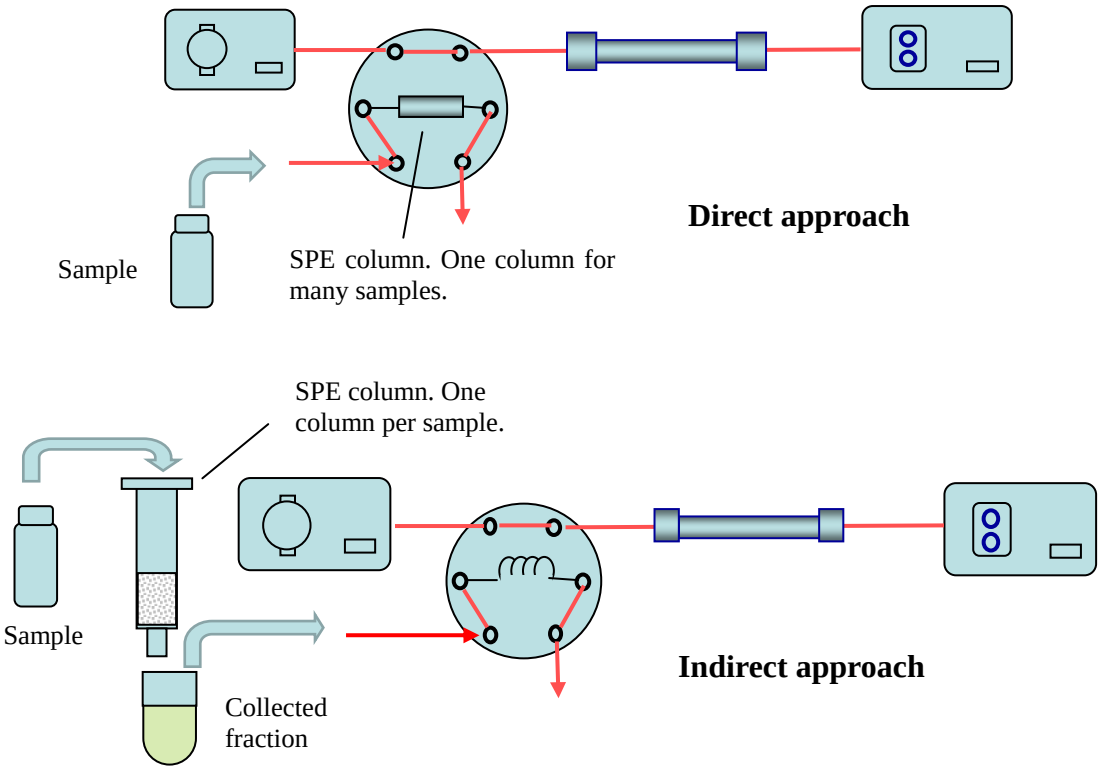
PromoChrom Technologies

Two-tier online solid phase extraction and large volume sample introduction for HPLC and LC-MS analysis using SPE-04 online SPE system

Integration of solid phase extraction with HPLC and LC-MS

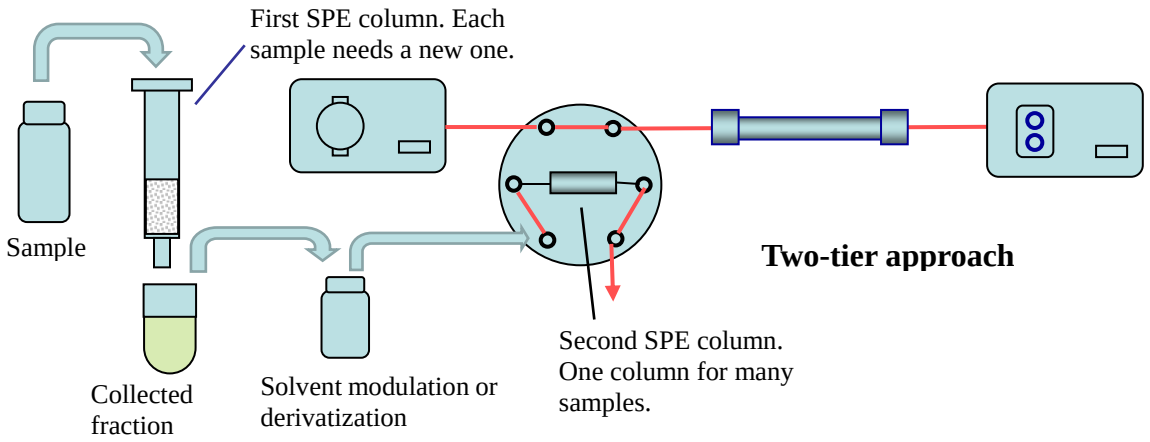
Integration of solid phase extraction (SPE) or column cleanup with liquid chromatographic (LC) analysis may take two approaches: 1) Direct coupling, in which a sample loaded in a cleanup column is completely transferred to the analytical column using a switching valve; 2) Indirect coupling, in which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



Using the direct approach can achieve high sensitivity and fast sample throughput since the entire cleaned up sample is used for final analysis and the procedure is simple. For example, in the analysis of trace pollutants in drinking water using offline SPE, the sample volume is normally around 500 mL.

The collected fraction is concentrated to 1 mL and an aliquot of 20 uL is used for LC analysis. This means that effectively, only 10mL from the 500 mL sample is used for LC analysis. If an online SPE is directly coupled with a LC, a 10 mL water sample can achieve the same sensitivity and the time for sample extraction is reduced from 2 hours to 10 minutes. The problem with the direct approach is finding a suitable SPE column that is compatible with the analytical column (to avoid peak broadening) and can be used for many samples, as it is not easy to change an SPE column that is fixed to a high pressure switching valve. On the other hand, the indirect approach can avoid this problem as the SPE column is decoupled from the HPLC column and only a very small volume is injected into the HPLC. The disadvantage of the indirect approach is with the sensitivity. Generally speaking, the direct approach is suitable for analysis that requires high sensitivity (such as at ppb or ppt level) and the sample matrix is clean, whereas the indirect approach is suitable for samples of more complex matrices and less demand on sensitivity.

PromoChrom hereby introduces a two-tier online SPE approach to overcome the disadvantages and make use the advantages of the two approaches described above. In a 2-tier online SPE, a sample is first extracted using a 3-mL or 6-mL SPE column like in offline SPE. The collected fraction (a portion or all) is then mixed with one or two solvents so that it can be trapped by the second SPE column (solvent modulation). The fraction is then loaded onto the second SPE column fixed on a switching valve and followed with more cleanup. All the trapped sample is then transferred to the LC column for final analysis.



The first SPE treatment is used to remove most of the interference. The column used can be a type of C18, ion exchange, or mixed mode. The second SPE column is used for enrichment and further cleanup. For example, the 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.

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 can also perform normal sample injection, and online derivatization with controlled temperature. In the present application note, a SPE-04 system is used to perform analysis of chloramphenicol in honey and tap water.

The HPLC coupled with SPE-04 is an Agilent 1100 system with a binary pump and a UV detector.

Materials and methods

The chloramphenicol standard was from Sigma-Aldrich 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, C18) from PromoChrom. The HPLC column was a PromoChrom's PCTsil C18 column (4.6X200mm, 5 um) or a Waters Nova-Pak C18 column (3.9X150 mm, 5 um). Flow rate was 1.5 mL/min. The detection was at 278 nm. Three gradient elution programs were used for HPLC analysis:

Program A: Methanol and water as mobile phase. Increase methanol from 10 to 70% over 3 minutes, hold for another 3 minutes, then return to 10% within 1 minute.

Program B: Acetonitril 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.

Program C: Acetonitril and water as mobile phase. Increase acetonitrile from 10 to 80% over 3 minutes, hold for another 4 minutes, then return to 10% within 1 minute.

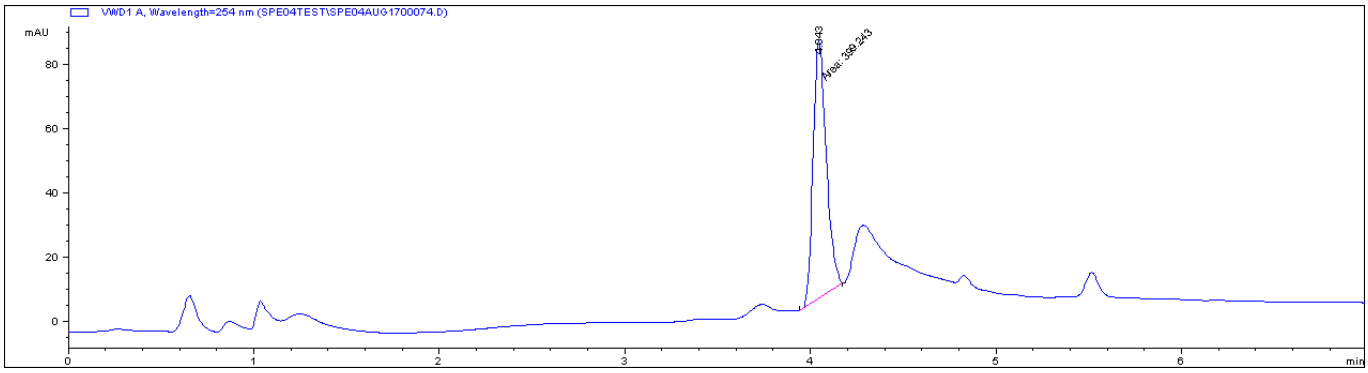
The honey used for experiment was named as "pure natural honey" by McCormick Canada. The tap water was collected in the area of Surrey area of British Columbia Province, which was originated from melted snow.

Results and discussion

1. Analysis of chloramphenicol in tap water using direct online SPE

Since the sample matrix is clean, direct approach was used for this analysis. Tap water was spiked

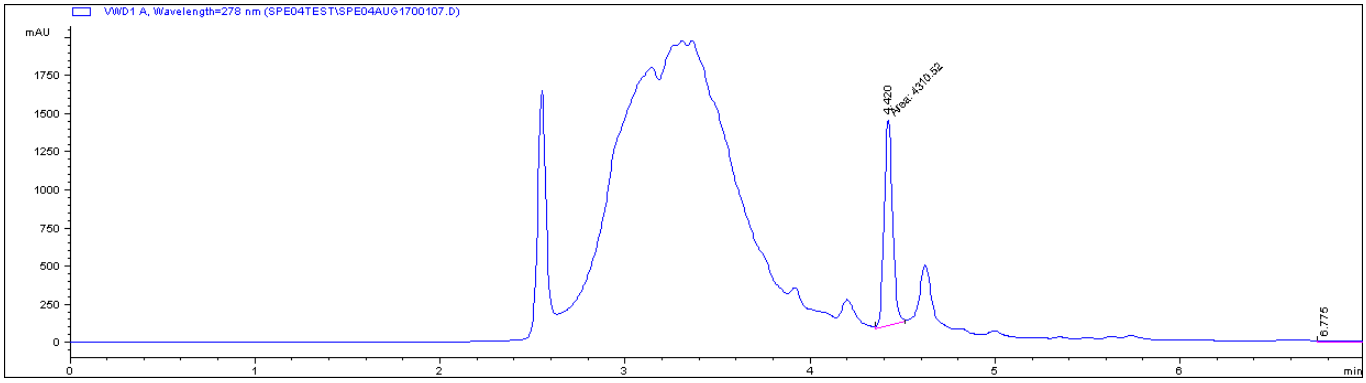
with chloramphenicol at 40 ppb level. A 10 mL sample was loaded to the SPE column 2 at 4 mL/min. After washing the column using 7.5 mL 10% methanol in water, the trapped sample was introduced into the LC column (Nova-Pak C18). Program A was used for the HPLC elution. Below is a chromatogram.



The time for one sample was 10 minutes for online SPE treatment. Since the HPLC analysis was performed in parallel with the SPE treatment and it was faster than the SPE process, the processing time for one sample is also 10 minutes. Based on the peak height, the detection limit is estimated as 2 ppb. Although increasing sample volume can increase the peak height, the background will limit the improvement of the detection limit. To achieve better sensitivity, a further purification method or a MSD need to be used.

2. Analysis of chloramphenicol in honey using direct online SPE

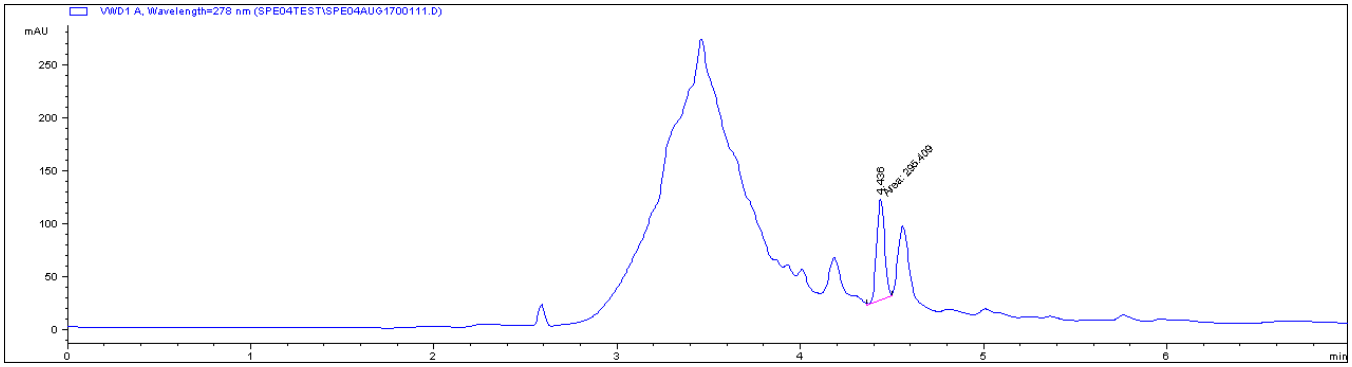
A 5 mL water solution containing 1.0 gram of honey and 4 ug chloramphenicol was loaded to the second SPE column (Trap N). The SPE column was washed with 5 mL water in the direction the same as the sample loading and then washed with 1.5 mL water in the reversed direction (to remove the particles). The 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. The estimated detection limit based on the peak height is 0.2 ppm. The processing time for one sample is 10 minutes.



After running 20 samples, the SPE column began to show blockage. To achieve better sensitivity and longer lifetime of the SPE column, the wash procedures need to be optimized. A treatment using liquid/liquid partition or an offline SPE prior to the online SPE should also help to extend the lifetime of the SPE column and to improve the detection limit. The following experiment demonstrates the benefits of using two-tier approach in extending the lifetime of the SPE column and improving the sensitivity.

3. Analysis of chloramphenicol in honey using two-tier online SPE

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. A 0.5 mL aliquot of the fraction (equivalent to 0.5 gram of honey) was mixed with 1.2 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 transferred to the HPLC. Gradient elution program B and a PCTsil C18 (4.6X200 mm) was used for the analysis. Below is a chromatogram of the sample.



Below is the detailed method for SPE-04 instrument:

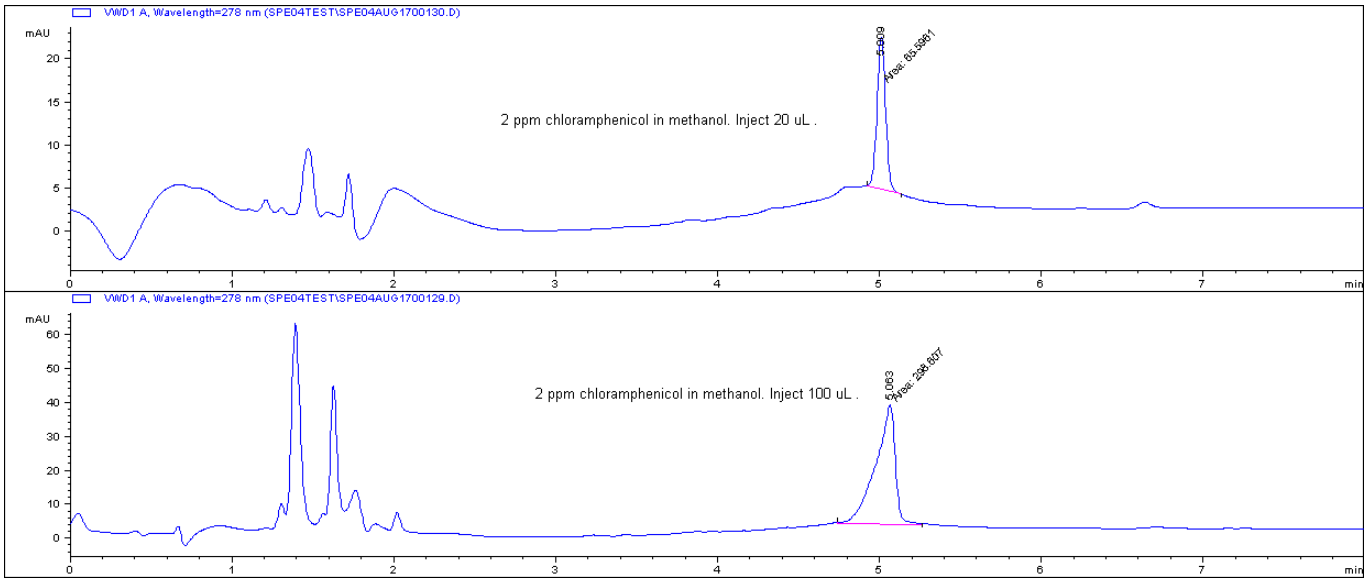
No.	Action	Flow rate	Volume	Remarks
1	Elute 1	5	2.5	Use 2.5 mL methanol to elute the first SPE column
2	Add samp	5	4.0	Add 4.0 mL sample to the first SPE column
3	Elute 2	5	7.5	Use 7.5 mL water to elute the first SPE column
4	Elute 1	5	0.1	Change elution solvent to methanol
5	Collect	5	1.0	Continue elution with methanol and collect 1 mL fraction
6	Mix Frac	0	1.2	Mix 0.4 mL fraction with 1.2 mL water in the mixing chamber
7	Load Mix	4	1.5	Load 1.5 mL mixed solution to the second SPE column
8	Wash 2	4	0.5	Use 0.5 mL water to back flush the second SPE column
9	Inject	1	1	Inject the trapped fraction to HPLC and trigger start LC analysis
10	Wait	1	2.0	Wait for 2 minutes to allow all the sample transferred to LC

In comparison with the chromatogram using direct online SPE, this two-tier approach has reduced the background considerably. The detection limit also improved due to reduced background interference. It is estimated as 0.05 ppm. The lifetime of the second SPE column was extended significantly. One SPE column worked well with no blockage or loss of trapping capability throughout 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).

4. Large volume sample injection using SPE-04

It is a normal practice in 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 the injection volume is too large, peak broadening will reduce the separation efficiency and reduce the sensitivity. Using the online solvent modulation function and the second SPE column in SPE-04 for enrichment, such adverse effect can be avoided when the injection volume is large and/or the sample solvent is very different from the LC mobile phase.

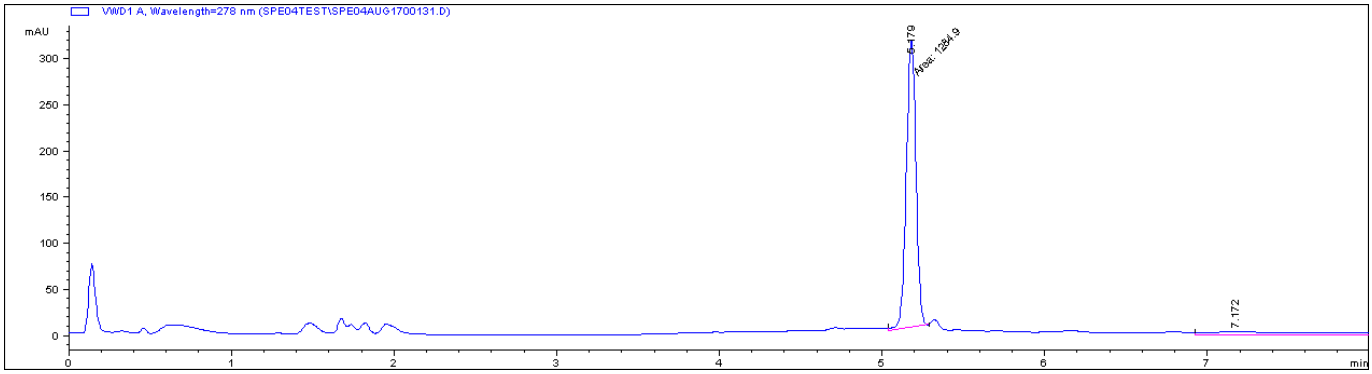
The present experiment compares the introduction of a methanol solution containing 2 ug/mL chloramphenicol into the LC column using a 20 uL sample loop and a 100 uL sample loop vs. using the second SPE column. The elution program C and a PCTsil C18 (4.6X200 mm) column was used for LC separation. The initial composition of the mobile phase was acetonitrile + water (10:90). It was very different from the sample solution. The following two chromatograms were obtained using 20 uL sample loop and 100 uL sample loop respectively:



As shown in the above chromatograms, a 20 uL sample volume gave a good peak shape despite the difference in solvent. When the injection volume was increased to 100 uL , the peak broadening

became very obvious.

In the case of 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. The SPE column was washed with 0.5 mL water to remove the possible particles from the sample and then the trapped sample was transferred to the HPLC. As shown in the chromatogram below, a 500 uL sample here can generate a peak as good as a 20 uL injection using a sample loop.



In trace analysis, further concentration and solvent exchange are often necessary after sample cleanup using SPE or QUECHERS. Such step can be time consuming and easy to generate errors on analytical results. The large volume introduction approach of SPE-04 can avoid these problems.

5. Discussion

This application note uses the analysis of chloramphenicol to demonstrate the benefits of online SPE. In the two-tier online SPE experiment, both the first SPE column and the second SPE column were of C18 type. For better cleanup, different columns such as an ion exchange column or an affinity column may be used as the first SPE column. A more careful optimization in washing procedures and a more selective detector should also improve the detection limit considerably.

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