Integration of sample preparation with chromatographic analysis

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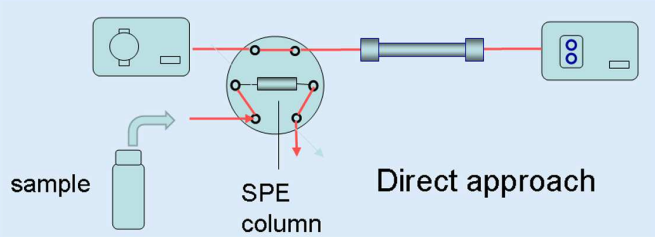
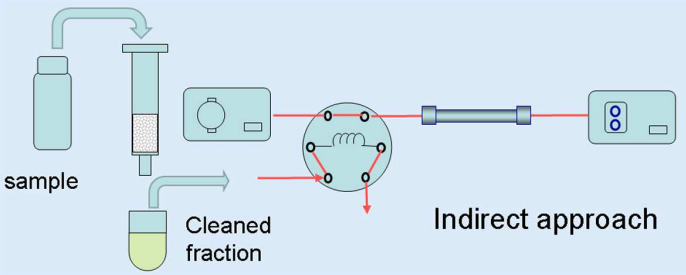
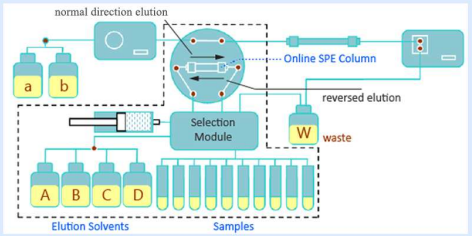
Integration of solid phase extraction or column cleanup with chromatographic analysis may take two approaches: 1) Direct coupling, in which a sample loaded in a cleanup column is completely transferred to the analytical column using a switching valve; 2) Indirect coupling, in which samples are treated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



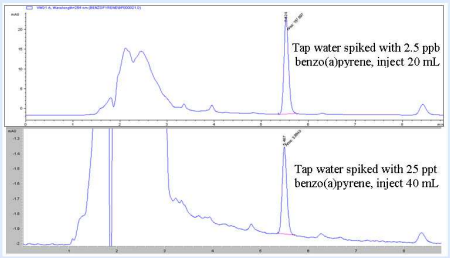


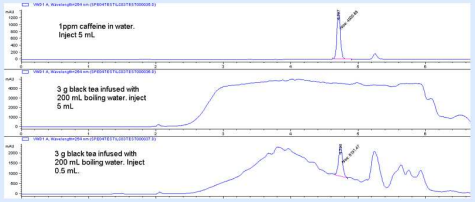
Using direct approach can achieve high sensitivity and fast sample throughput since all the treated sample is used for final analysis and the procedure is simple. For example, in analysis of trace pollutants in drinking water using offline solid phase extraction (SPE), the sample volume is normally 500 mL. The collected fraction is concentrated to 1 mL and an aliquot of 20 uL is injected for LC analysis. From the 500 mL sample extracted, only 10 mL is used for final analysis. 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. The problem with the direct approach is to find a suitable SPE column that is compatible with the analytical column and can be regenerated easily. 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. Compared to offline SPE, online SPE can reduce human involvement in sample handling and increase data reproductibility. 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 relatively clean, whereas the indirect approach is preferred for samples of more complex matrix and less demand on sensitivity. This poster will present the instrumentations for the two online approaches and their applications.

LC-03 online SPE for direct coupling with LC



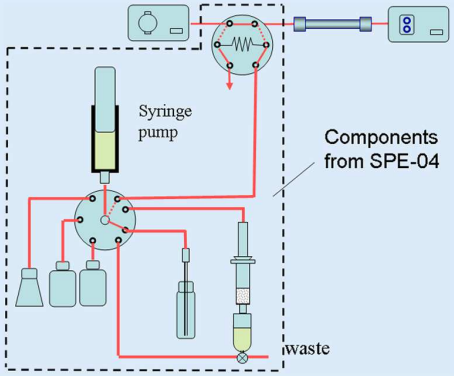
A special feature of LC-03 is that the SPE column can be washed from two directions. It is very efficient to remove trapped particles by washing the SPE column in the reverse direction. It also helps to obtain good peak shape for very retentive compounds. Thanks to this feature, one SPE column can be used for at least 50 samples and in most cases the sample does not need filtration. Below are two examples. The left is for benzopyrene and the right is for caffeine.



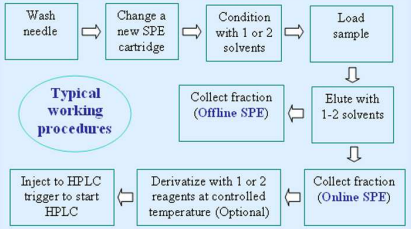


For benzopyrene: LC column, PromSil C18 4.6X250 mm, 5 um particle. Methanol as mobile phase at 1.5 mL/min. SPE column, TrapN 4.6X10mm. Detection at 254 nm.
For caffeine: LC column, PromSil C18 4.6X200 mm, 5 um particle. Gradient from 10% to 70% methanol in 3 minutes at 1.5 mL/min. SPE column, TrapN 4.6X10mm. Detection at 254 nm.

SPE-04 online SPE for indirect coupling with LC



SPE-04 can perform both online and offline SPE. In addition, it has a function for online derivatization at controlled temperature .



Application example: **analysis of hormone in plasma**

1) Dilute sample with 1% phosphoric acid at 1:5 ratio.
2) Precondition a 3-mL/200-mg C18 SPE column with 2 mL methanol followed by 2 mL water.
3) Load 2 mL sample and wash with 4 mL water+methanol (80:20).
4) Elute SPE column using methanol and collect 1 mL fraction.
5) Derivatize the fraction with dansyl chloride at 60 degrees.
6) HPLC analysis using a PCTsil C18 column and UV or fluorescence detection.