Advantages and Limitations of HPLC in Environmental Analysis

J. F. Lawrence
Food Research Division, Health Protection Branch, Ottawa, Ontario, Canada K1A 0L2

Key Words
HPLC
PAH (polycyclic aromatic hydrocarbons)
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Organo-metallics
Sulfite
Headspace-HPLC

Summary
The application of HPLC to environmental analysis is often hindered by difficulty not experienced in other areas of analysis. Usually the components being determined are at parts per million levels or less and are usually in sample matrices that can yield many interferences. In order to develop successful methodology the prime requirements for an HPLC system are column efficiency and the sensitivity and selectivity of the detection system. In this presentation, examples are given to illustrate how HPLC can be used to advantage by comparing it to gas chromatographic (GC) methods and even TLC methods. For many classes of compounds, such as halogenated hydrocarbons (pesticides, PCB's, dioxins), the GC methods may be preferred. However, for polycyclic aromatic hydrocarbons (PAH) HPLC with fluorescence detection has proven to be excellent for trace environmental analysis. Comparisons of HPLC with TLC for aflatoxins and with bioassays for paralytic shellfish toxins are made. Novel combinations such as headspace-HPLC analyses for SO₂ and HPLC-AA for organometallic compounds are discussed.

Introduction
High performance liquid chromatography (HPLC) has become a widely used technique for the analysis of many substances including both organic and inorganic compounds in a large variety of sample types. Environmental analyses, however, often require special HPLC conditions. The concentrations to be determined are often in the parts per million range or less. At these levels, the detector characteristics become very important. Both sensitivity and selectivity are required. The refractive index detector, for example, is widely used for HPLC applications where extremely low level detection is not required. This detector is unsuitable for trace environmental analysis.

Sensitivity and Selectivity
Sensitivity alone may not be enough to enable a determination to be made. Fig. 1 shows chromatograms of a pesticide, asulam, in a food extract at 1.0ppm run at different UV absorption wavelengths [1]. Although the compound is detectable at all three wavelengths it can be seen that at 254nm the selectivity is poor resulting in interferences from the food matrix. At the wavelength maxima for asulam, 268nm, the chromatogram has improved. However, best selectivity was found at 280nm, and, in fact, would be the wavelength of choice for routine analysis.

Fig. 1
Chromatograms of asulam in whole wheat flour extract (at 1.0μg/g) with different detector wavelengths.
the equivalent of 1 mg of sample was injected for the GC
analysis (which employed an electron capture detector)
compared to 200 mg required for the HPLC-UV absorption
determination. This illustrates the much better sensitivity
of the GC system compared to the HPLC one. However,
the latter provided better results based on signal: back-
ground ratio, because of the superior selectivity of the
detection system.

The disadvantage of poor sensitivity with good selectivity
is that large amounts of sample extract must be injected
which can lead to shortened column life. Normally it is
best to inject as little sample as possible while using the
most sensitive detector setting that still gives a good bas-
line.

Applications

There are many areas in environmental analytical chemistry
where HPLC is less suited than, for example, capillary GC.
The latter uses columns (30 m) which can have efficiencies
of around 100,000 theoretical plates whereas commonly
used HPLC columns have only about 10,000 plates or less.
For compounds such as halogenated hydrocarbons (pesti-
cides, PCB’s, dioxins) which are thermally stable, the high
separation efficiencies and sensitive detection by electron-
capture or mass spectrometry make capillary GC the
method of choice.

However, in other areas, HPLC is clearly advantageous. It
is particularly suited to the determination of polycyclic
aromatic hydrocarbons. Many of these compounds have
intense native fluorescence and can be readily detected in
the picogram range [3]. Fig. 3 shows a separation of eight
PAH standards. Some of these compounds could be easily
detected at 20 pg. Table I compares detection limits ob-
tained by HPLC-fluorescence with GC-MS with selected
ion monitoring. It can be seen that the HPLC method is
somewhat more sensitive. Fig. 4 shows chromatographic
results using HPLC-fluorescence for several food types.
It can be seen that for some samples many unidentified
peaks appear. From the sample preparation procedure and
the HPLC conditions it is likely that these substances are
other PAH related compounds because of their non-polar
nature and from the fact that they fluoresce.

Another example of the use of HPLC with fluorescence
detection is for the determination of aflatoxins in foods.
These fungal mycotoxins are strong carcinogens and thus
methods must be available to detect them in contaminated
foods, (usually nuts and grain type products) at low ng/g
levels. Fig. 5 shows HPLC results of work done by Tartar
et al. [4] of the Canadian Health Protection Branch for four
aflatoxins spiked in peanuts. The chromatogram represents
aflatoxin levels of 0.5 and 1.0 ng/g in shelled peanuts.
Aflatoxins B1 and G1 must be derivatized to B2a and G2a
in order to have adequate fluorescence. While the HPLC
detection limits are very good it should be pointed out
that this technique is not necessary for rapid screening
since direct TLC methods are equally as sensitive capable
of detecting sub ng/g levels of these toxins without the use
of expensive instrumentation. The HPLC method of course
would be preferred for quantitation.