Chromatography Products

Environmental Applications

Reduce Acetonitrile Dependence

by Analyzing Polycyclic Aromatic Hydrocarbons with Methanol-Based Mobile Phase

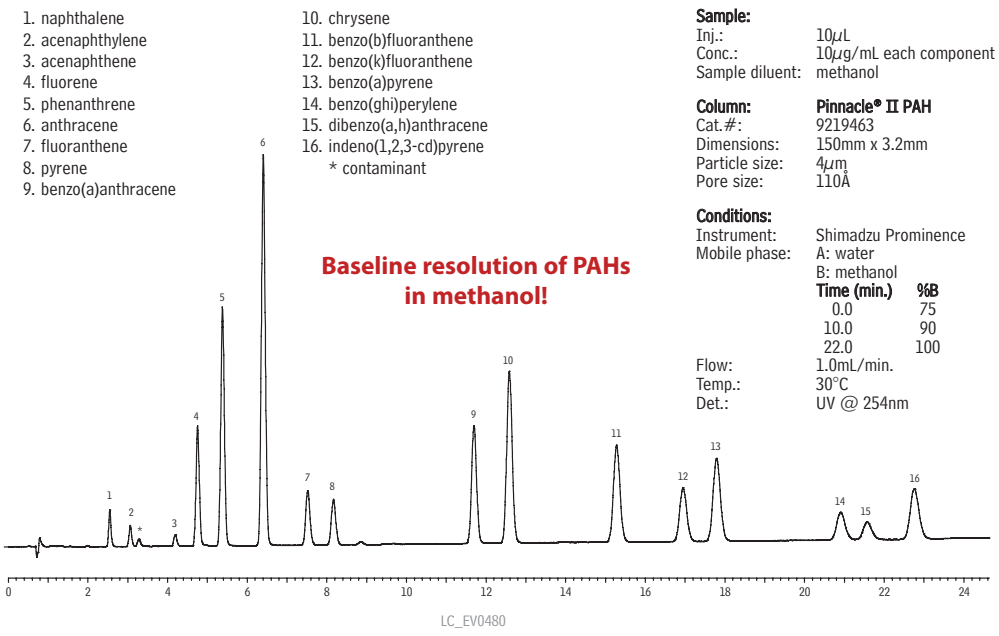
The recent acetonitrile shortage has resulted in limited availability and significantly increased solvent costs. In response, many labs are interested in alternate mobile phases for HPLC methods. Here we demonstrate an effective method for analyzing polycyclic aromatic hydrocarbons (PAHs) using methanol, instead of acetonitrile, resulting in a cost savings of approximately US$40 per liter. Baseline resolution of 16 target PAHs was easily obtained in approximately 23 minutes using a Pinnacle® II PAH column with a methanolic mobile phase.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants found in air, water, soil, and sludge. PAHs are an increasing human health concern, as this group of chemicals includes several known or suspected carcinogens. Analysis of PAHs often is done by high pressure liquid chromatography (HPLC) because it provides a higher degree of selectivity between structural isomers than gas chromatography (GC). Chromatographic separation of these isomers is critical, because they cannot be distinguished by a mass spectrometer. Acetonitrile is commonly used in the mobile phase when analyzing PAHs by HPLC; however, the recent acetonitrile shortage has limited its availability and significantly increased analytical costs. Here we detail conditions using a methanolic mobile phase and a Pinnacle® II PAH column that result in baseline resolution of 16 priority PAHs, offering labs a cost-effective alternative to using acetonitrile.

While switching to alternate solvent systems can be an attractive way to reduce costs, several points must be considered first, including: mode of separation, detector, and analyte compatibility. Here, reverse phase chromatography is used, so a polar mobile phase solvent, such as methanol, must be used with a nonpolar stationary phase. Since an ultraviolet (UV) detector is used, the UV cutoff of the solvent also must be considered. PAHs are analyzed at 254nm, so methanol can be used without producing interference, because its UV cutoff is 205nm (acetonitrile is 190nm). Finally, preliminary injections should be performed to ensure that the solvent does not react with target analytes in a way that causes undesirable effects, such as peak tailing, degradation peaks, or poor response. These points should always be considered when evaluating an alternate mobile phase solvent.

Figure 1 Reduce acetonitrile costs by analyzing PAHs on Pinnacle® II PAH columns with a methanolic mobile phase.



Analytical Reference Materials

SV Calibration Mix #5 / 610 PAH Mix

(16 components)

acenaphthene	chrysene
acenaphthylene	dibenzo(a,h)anthracene
anthracene	fluoranthene
benzo(a)anthracene	fluorene
benzo(a)pyrene	indeno(1,2,3-cd)pyrene
benzo(b)fluoranthene	naphthalene
benzo(k)fluoranthene	phenanthrene
benzo(ghi)perylene	pyrene

2,000µg/mL each in methylene chloride, 1mL/ampul

cat. # 31011 (ea.)

610 PAH Calibration Mix A (16 components)

For HPLC/fluorescence detection.

acenaphthene	1,000µg/mL	chrysene	500
acenaphthylene	1,000	dibenzo(a,h)anthracene	500
anthracene	1,000	fluoranthene	500
benzo(a)anthracene	500	fluorene	1,000
benzo(a)pyrene	500	indeno(1,2,3-cd)pyrene	500
benzo(b)fluoranthene	500	naphthalene	1,000
benzo(k)fluoranthene	500	phenanthrene	500
benzo(ghi)perylene	500	pyrene 500	

In methylene chloride, 1mL/ampul

cat. # 31264 (ea.)

610 PAH Calibration Mix B (16 components)

For HPLC/UV detection.

acenaphthene	1,000µg/mL	chrysene	100
acenaphthylene	2,000	dibenzo(a,h)anthracene	200
anthracene	100	fluoranthene	200
benzo(a)anthracene	100	fluorene	200
benzo(a)pyrene	100	indeno(1,2,3-cd)pyrene	100
benzo(b)fluoranthene	200	naphthalene	1,000
benzo(k)fluoranthene	100	phenanthrene	100
benzo(ghi)perylene	200	pyrene 100	

In methylene chloride:methanol (1:1), 1mL/ampul

cat. # 31455 (ea.)

EPA Method 8310 Surrogate Standard

decafluorobiphenyl

1,000µg/mL in acetonitrile, 1mL/ampul

cat. # 31842 (ea.)

PATENTS & TRADEMARKS

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Procedure

A mixed standard containing 16 priority PAHs at 10µg/mL each was prepared in methanol. Note that it is important to prepare samples and standards in mobile phase or a compatible solvent, otherwise poor peak shape may be observed. The mixed standard was analyzed using a mobile phase gradient consisting of HPLC grade methanol and deionized water under constant flow conditions (1mL/min.). Since methanol is a weaker mobile phase than acetonitrile, a slightly higher starting percentage was used in order to achieve the desired resolution (75% methanol vs. 65% acetonitrile in typical methods). A Pinnacle® II PAH column (150mm x 3.2mm, 4µm) was chosen for this analysis, because it has a unique selectivity and is known to perform well for PAHs. All analyses were performed on a Shimadzu Prominence HPLC equipped with a UV detector set at 254nm. Column temperature was maintained at 30°C.

Results

All 16 PAHs were baseline resolved on the Pinnacle® II PAH column using a methanolic mobile phase (Figure 1). One elution order change was noted: when using acetonitrile, dibenzo(ah)anthracene elutes before benzo(ghi)perylene, however the opposite elution order is observed when using methanol. Total analysis time was approximately 23 minutes, which is slightly longer, but comparable, to common acetonitrile-based methods. Since acetonitrile is currently much more expensive than methanol, this methanol-based procedure offers labs an opportunity to considerably reduce solvent costs, while maintaining complete resolution of all target compounds.

Conclusion

The goal of this experiment was to establish conditions for analyzing PAHs using an alternate mobile phase in order to reduce acetonitrile use and its associated costs. Using a methanol-based mobile phase and a Pinnacle® II PAH column, all 16 target PAHs were baseline resolved in 23 minutes. The use of alternate solvent systems—such as the methanol-based PAH analysis shown here—should be considered by labs interested in reducing acetonitrile costs.

HPLC Columns

Pinnacle® II PAH Columns

Physical Characteristics:

particle size: 4µm, spherical
pore size: 110Å
endcap: fully endcapped
pH range: 2.5 to 10
temperature limit: 80°C

Length	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
4µm Columns			
50mm	9219452	9219453	9219455
100mm	9219412	9219413	9219415
150mm	9219462	9219463	9219465
200mm	9219422	9219423	9219425
250mm	9219472	9219473	9219475

To order one of these columns with a Trident™ Integral Inlet Fitting, add "-700" to the catalog number for the column. (Nominal additional charge)

For guard cartridges for these columns, visit **www.restek.com**

Lit. Cat.# EVAN1164

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