Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography A, 1142 (2007) 164–171
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Routine determination of benzo[*a*]pyrene at part-per-billion in complex industrial matrices by multidimensional liquid chromatography

C. Champmartin *, P. Simon, Ph. Delsaut, M. Dorotte, B. Bianchi

Institut National de Recherche et de Sécurité, Avenue de Bourgogne, 54501 Vandoeuvre Cedex, France

Received 22 September 2006; received in revised form 11 December 2006; accepted 14 December 2006
Available online 27 December 2006

Abstract

A rapid and selective high performance liquid chromatography (HPLC) method using a column-switching technique has been developed for the determination of benzo[*a*]pyrene in complex mixtures containing polycyclic aromatic hydrocarbons. The diluted sample is directly injected into the chromatographic system without pre-treatment. The purification is performed on-line using three cleaning columns filled with various stationary phases. The sample preparation, a simple dilution, and the analysis time do not exceed 45 min. The method developed was used to analyze industrial products such as oil, bitumen, etc. and was compared with an off-line method requiring treatment and extraction steps before the analysis.

Keywords: Liquid chromatography; Benzo[*a*]pyrene; Column-switching; Petroleum products; Coal-derived products

1. Introduction

In the field of occupational hygiene, it is necessary to analyze very complex mixtures containing polycyclic aromatic hydrocarbons (PAH) such as mineral oils, coal tars, inks, carbon blacks, etc. Indeed, some PAH, and particularly benzo[*a*]pyrene (BaP), are mutagenic and carcinogenic, the quantification of these PAH in the matrices is thus of prime importance as it can constitute a supplementary information to biological and atmospheric monitoring for the assessment of occupational exposure to PAH. BaP, one of the known carcinogens and probably the most thoroughly studied, has therefore been selected as a leading substance to be determined [1]. In France, in the 1970's, the National Health Insurance Fund (CNAM) recommended a value of 150 ng/m^3 for atmospheric BaP [2].

Among other various chromatographic techniques (gas chromatography: GC, gas chromatography–mass spectrometry: GC–MS, thin-layer chromatography: TLC) [3–6], high performance liquid chromatography (HPLC) [6–11] is widely used in many laboratories for the determination of PAH in the atmosphere, in industrial products and in environmental samples. However, due to the large number of components in complex samples and the low concentrations of BaP in some matrices (ppm to ppb range), these samples may be difficult to analyze by conventional methods. These methods use generally cumbersome and time-consuming off-line purification procedures. The use of column-switching techniques minimizes the manual treatment of samples considerably and consequently reduces the overall analysis time. It also leads to a reduction in potential interferences due to the presence of long chain hydrocarbons (e.g. paraffins and olefins), aromatics and polar compounds or the presence of additives.

Considering the experience of our laboratory in the field of multidimensional liquid chromatography [12,13], a method has been developed that allows rapid isolation and quantitative determination of BaP in various and complex industrial matrices by column-switching HPLC with minimum sample manipulation and preparation. It can easily be modified for the dosage of other individual PAH such as pyrene. The sample is purified on-line under isocratic elution conditions using the heart-cutting technique and a mini-column installed in the injection loop permits a possible sample concentration. This on-line method was compared with an off-line method requiring liquid phase and solid phase extraction pre-treatments before analysis by HPLC.

* Corresponding author. Tel.: +33 383502000.
E-mail address: catherine.champmartin@inrs.fr (C. Champmartin).

0021-9673/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2006.12.082

2. Experimental

2.1. Chemical and reagents

All chemicals were of analytical reagent grade. The water was purified by passing it through a Milli-Q treatment system (Millipore, Bedford, MA, USA). Chromatographic grade methanol (MeOH), 2-propanol and tetrahydrofuran (THF) were obtained from Merck (Darmstadt, Germany), acetonitrile (ACN) and dichloromethane (CH_2Cl_2) from S.D.S. (Peypin, France) and cyclohexane, dimethylformamide (DMF), ethanol (EtOH), toluene and benzene from Prolabo (Fontenay sous Bois, France).

The MeOH and ACN used to prepare the mobile phases of the on-line method must be freshly distilled using a rotary evaporator in order to eliminate impurities that cause interferences and serious disturbance of the baseline.

Benzo[*a*]pyrene (purity: 99%) was purchased from Radian Corporation. A mixture of 16 polycyclic aromatic hydrocarbons (610 M) was obtained from Supelco. A reference oil sample (Standard Reference Material 1582, Petroleum Crude Oil) was provided by LGC Promochem SARL (Molsheim, France). Helium Alphagaz He (1) (Air Liquide, France) was used to degas the mobile phases.

2.2. Apparatus

2.2.1. On-line method

The HPLC system consisted of the following: two chromatographic pumps, model LC10-ATVP (Shimadzu, Kyoto, Japan); four automated switching valves (six-way valves two positions, Rheodyne, Berkeley, CA, USA), one being equipped with a miniature precolumn loop; a model RF10AXL fluorescence detector (Shimadzu, Kyoto, Japan) set to 296 nm for excitation and 405 nm for emission for BaP analysis, equipped with a Xenon lamp (OSRAM). The entire system was controlled by a SCL10VP controller and the CLASS VP program (Shimadzu, Kyoto, Japan). The output signal was recorded with a Datajet Integrator (Thermo Electron, France) or a microprocessor unit. The purification columns and the analytical column were kept at constant temperature in a CT0 10 AS VP thermostated column oven (Shimadzu, Kyoto, Japan). The mobile phases were degassed either with helium or with a Degasys Populaire in-line degazer (Uniflows, Tokyo, Japan). A rotary evaporator (Büchi, Switzerland) was used to distil the solvents of the mobile phases.

2.2.2. Off-line method

The HPLC system consisted of a Model 880 PU chromatographic pump (Jasco, Tokyo, Japan) combined with a model 880-02 gradient unit (Jasco) and a model 801 SC system controller (Jasco); a model 7410 sample injector (Rheodyne) equipped with a 5-μl loop; a model RF551 fluorescence detector (Shimadzu, Kyoto, Japan) set to 296 nm for excitation and 405 nm for emission for BaP analysis, equipped with a Xenon lamp (OSRAM). The output signal was recorded with a Waters 746 Data Module Millipore integrator. The analytical column was kept at constant temperature in a CT0 10 AS VP thermostated column oven (Shimadzu, Kyoto, Japan). The mobile phases were degassed with helium.

A rotary evaporator (Büchi, Switzerland) was used to evaporate the solvents during the treatment steps of the samples.

2.3. Columns

2.3.1. On-line method

The precolumn loop was a 2 cm × 0.21 cm i.d. stainless-steel guard column cartridge (Upchurch Scientific, Oak Harbor, WA, USA) filled with 40 μm Bondesil C_1 (Varian). Purification columns C1 and C2 were 5 cm × 0.46 cm stainless-steel tubes packed with 5 μm Kromasil C_1 (Eka Nobel), for C1 and with 5 μm regardless of Nucleosil NO_2 or Polygosil NO_2 (Macherey-Nagel, Düren, Germany) for C2. The purification column C3 was either a 3.5 cm × 0.21 cm stainless-steel tube packed with 5 μm Vydac 201 TPB (The Separation Group) or three guard columns 1.3 cm × 0.21 cm packed with Vydac 201 TP (Interchim). Analytical column C4 was a stainless-steel tube (15 cm × 0.32 cm) packed with Uptisphere 5PAH (Interchim).

2.3.2. Off-line method

The analytical column was a stainless-steel tube (10 cm × 0.46 cm) packed with Chromspher 3PAH Chromsep SS (Varian).

With the exception of the precolumn loop, which is dry filled, and both the guard columns and the Chromspher column which are commercially available, all the columns were packed in the laboratory at 4×10^7 Pa using a mixture of 95% ethanol–2-propanol–toluene (1:1:1, v/v/v) as slurry solvent, followed by methanol and then by water as displacement liquid.

However, these columns are either directly commercially available or can be tailor-made by the suppliers.

2.4. Analytical procedure

2.4.1. On-line method

Schematic diagrams of the switching system are shown in Fig. 1 and the timetable of the procedure is given in Table 1. In this table, time values are given as an example. BaP retention times must be determined on each purification column.

The mobile phase (E1) used for the purification columns (C1, C2 and C3) was a mixture of methanol–water–acetonitrile (70:25:5, v/v/v). The mobile phase (E2) used for the analytical column (C4) was a mixture of the same solvents (6:8:86, v/v/v). They were all eluted in isocratic mode at 0.5 ml/min. When helium was used to degas the mobile phases, prior to analysis, the mobile phases were degassed with helium for 5 min and kept under a helium atmosphere during the analysis.

Prior to each injection, the precolumn loop was manually preconditioned with 200 μl of water, and a variable volume sample (10–40 μl) was then injected into the chromatographic system with an HPLC glass syringe. After the sample had been loaded, the precolumn loop was flushed with 200 μl of a mixture of methanol–water (40:60, v/v) at about 2 ml/min to remove undesirable impurities (step 1).This step can be automated using appropriate equipment. The next steps of the column-switching