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Analysis of Polycyclic Aromatic Hydrocarbons by Supercritical Fluid Chromatography Using an Improved Binary Gradient as Mobile Phase

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Este trabalho descreve um método de determinação de nove hidrocarbonetos aromáticos policíclicos por cromatografia com fluido supercrítico. A fase móvel utilizada foi um gradiente multietapa de adição de cicloexano ao CO_2 supercrítico: 0% (0 a 16 min), 4% (17 a 21 min) e 10% (22 a 29 min). O equipamento utilizado contava com uma coluna C_{18}, um detector UV a 254 nm e um sistema especial de injeção que eliminava o solvente entre múltiplos depósitos, aumentando a sensibilidade da análise. O método é linear e reprodutível, tem uma boa resolução e seu limite de detecção é de 20 ng. Uma aplicação à contaminação atmosférica é apresentada.

This paper describes a supercritical fluid chromatographic assay for nine polycyclic aromatic hydrocarbons. The binary mobile phase was composed of CO_2 and cyclohexane, with the latter applied as a multi-step gradient: 0% (0 to 16 min), 4% (17 to 21 min) and 10% (22 to 29 min). A C_{18} Vydac 201 TP column, a 254 nm UV detector, and a system which eliminated injected solvent, thereby permitting multiple sample accumulations prior to injection, were used. The method gives good resolution, shows a linear response, is reproducible and has a detection limit of 20 ng. An application to studies of vehicle emissions and ambient air is described.

Keywords: *polycyclic aromatic hydrocarbons, supercritical fluid chromatography*

Introduction

Combustion or pyrolysis of organic compounds, especially those with unsaturated, branched-chain or aromatic structures, produces many polycyclic aromatic hydrocarbons (PAH). Diverse sources, such as incinerators, transport fuels, tobacco smoke, urban heating systems, and industrial processes, contribute to the presence of such hydrocarbons in the environment. A specific and sensitive assay for PAH is needed, particularly because those in the gaseous or particulate phases of atmospheric pollution are thought to be carcinogenic.

Various methods are available for the assay of PAH such as gas chromatography (GC) combined with flame ionization[1-4] or mass spectrometry[5] detectors. Higher resolution can be obtained by using high-performance liquid chromatography (HPLC), coupled with UV[6-9] or spectrofluorimetric[10-12] detection. Good resolution is obtained using supercritical fluid chromatography (SFC) with flame ionization[13-16], mass spectrometry[17], chemiluminescence[18] or UV detection[19,20]. Analysis times are also short. The resolution and speed of SFC are attributable to the low viscosity of the mobile phase; the large diffusion coefficient of the analyte provides maximum efficiency and a linear velocity greater than that achieved with HPLC.

An improved PAH assay based on supercritical fluid chromatography with a packed ODS column was developed. A CO_2-cyclohexane binary gradient of supercritical fluid gave good separation of isomers such as benzo(k)fluoranthene and benzo(b)fluoranthene in a mixture of nine PAH considered to be markers of atmospheric pollution. A special multiple sample injection system permitted the detection limit to be extended at 254 nm. We describe the use of our method for the analysis of samples of vehicle emissions and ambient air in Paris (France).

Materials and Methods

Reagents

Analytical grade fluoranthene (F), pyrene (P), benz(a)anthracene (B(a)A), benzo(k)fluoranthene (B(k)F), benzo(b)fluoranthene (B(b)F), benzo(a)pyrene (B(a)P), dibenzo(a,h)anthracene (DB(ah)A), indeno(1,2,3-c,d)pyrene (I(123cd)P), and benzo(g,h,i)perylene (B(ghi)P were obtained from Aldrich. Standard solutions of these compounds were prepared in HPLC-grade acetonitrile (Merck) and/or dichloromethane (Carlo Erba). The mobile phase consisted of 99%-pure CO_2 (AGA) and HPLC-grade cyclohexane (Carlo Erba).

The assay was calibrated using a dilution series (0.5 to 5.0 mg/L) of a standard solution containing all the compounds; each solution was injected three times.

Reproducibility was tested by making ten injections of a solution containing: F(1.4 mg/L), P (2.4 mg/L), B(a)A (2.0 mg/L), B(k)F (3.6 mg/L), B(b)F (3.5 mg/L), B(a)P (3.2 mg/L), D(ah)P (3.5 mg/L), I(123cd)P (3.3 mg/L), and B(ghi)P (2.4 mg/L).

Equipment

The system comprised a Carlo Erba SFC 3000 chromatograph, modified by the addition of a valve (Tescom 26-1700), a UV spectrophotometer (UV SPD 6A, Shimadzu) equipped with a high-pressure cell, and a special two-valve (Valco N 6 W and a Rheodyne 7125) injection system equipped with a C_{18} precolumn [20 mm x 2.1 mm x 5 μm (HP)]. The chromatographic column used for analysis was C_{18} [Vydac 201 TP 54, 250 mm x 4.6 mm x 5 μm (The Separations Group)]. The system was equipped with a computer to calculate pseudo-critical parameters of the mobile phase mixtures.

Injection conditions

The injection system was assembled according to Majdalani[21] (Fig. 1). Both valves were set to position A to allow the application of the liquid sample to the precolumn using a 100.0 μL Hamilton syringe during elution of the chromatographic column with CO_2. When valve 2 was set to position B, a constant flow of helium passed through the precolumn and evaporated the sample solvent for a known

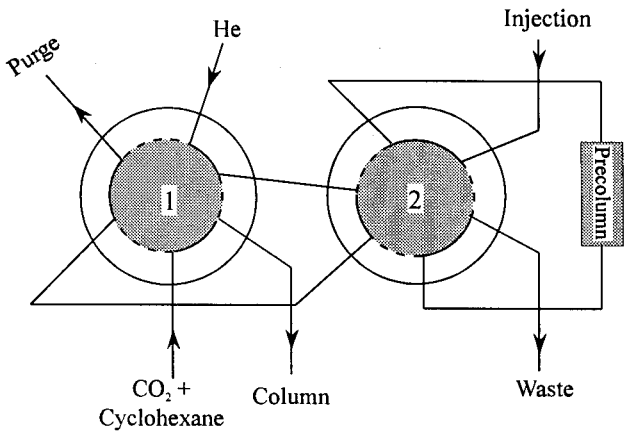

Figure 1. Diagram of the injection system. Position A: —; Positon B: - - - -

time (6 min for acetonitrile). Six applications of the liquid sample to the precolumn (valve 1 = A and valve 2 = A), and six evaporations of the sample solvent (valve 1 = A and valve 2 = B) were carried out. Finally, valve 1 was set to position B to introduce supercritical CO_2 into the precolumn and elute the samples towards the chromatographic column.

Soot extraction from vehicle emissions and air samples

This was done according to the French norm NF X 43-025, October 1988[22]. Soot was collected as follows: exhaust emissions were diluted with air (flow ratio, 0.015), using a 293 mm diameter Teflon-coated fiberglass Pallflex filter type T60A20 (Pall Corp.). Air samples were collected at a flow rate of 5 m^3/h using a Pallflex filter.

Fifty mg of particles from the filter were extracted (2 x 15 min) with 50 mL of dichloromethane under ultrasonic agitation, in a stoppered silanised glass bottle. The extract was then filtered (porous glass) and evaporated to around 500 μL under vacuum using a rotary evaporator at 35 °C.

Soot samples were further evaporated to 200 μL under nitrogen. The residue obtained was redissolved in 1.0 mL cyclohexane before purification. Air samples were evaporated to dryness before chromatography.

Purification of soot from vehicle emissions

Soot was purified to eliminate interfering polar polyaromatic compounds which coelute at the beginning of the chromatogram. The extract was applied to a microcolumn containing 0.5 g of silica (Mega Bond Elut -Varian). PAH were extracted under vacuum using cyclohexane and dichloromethane (5.0 mL, 50-50 v/v). Polyaromatic polar compounds were retained on the column. The eluant was evaporated to dryness under high purity helium, and the residue redissolved in 0.50 mL of acetonitrile before injection.

Calculations of solubility and pseudo-critical parameters of the CO_2-cyclohexane binary system

The solubility of a supercritical fluid is defined by the Hildebrand parameter[23]:

$$\delta = 1.25 \sqrt{P_c} \frac{\rho}{\rho_l} \quad (1)$$

where P_c is the critical pressure, ρ is the fluid density under given temperature and pressure conditions, and ρ_l is the density of the corresponding liquid.

In a binary fluid system, the solubility parameter, δ_m, can be estimated from the volumetric and thermodynamic properties of the gas or vapor as a function of three parameters: reduced temperature ($T_r = T_c$), reduced pressure ($P_r = P_c$), and the acentric factor ω [Lee correlation[24], $\omega = -\log P_c$ (at $T_r = 0.7$) - 1.00].

The binary interaction parameter, k_{12}, can be calculated using the correlation from Plöcker *et al.*[25]:

$$k_{12} = 0.9 + 0.02 \frac{T_{c,2} V_{c,2}}{T_{c,1} V_{c,1}} \quad (2)$$

where $T_{c,1}$ and $T_{c,2}$ are the critical temperatures of CO_2 and cyclohexane respectively, and $V_{c,1}$ and $V_{c,2}$ are their critical volumes.

We have been able to calculate the pseudo-critical pressure (P_c'), temperature (T_c') and density (ρ') of the mixture, according to Ptizer *et al.* [26-28], using the critical parameters of CO_2 and cyclohexane: P_c, T_c, ω (available from reference tables[29]); the molar fractions of the fluids in the mixture and k_{12}. The solubility parameters of the mixture, δ_m, were deduced from (1) (Table 1) using pseudo-critical values.

Results and Discussion

Supercritical fluid chromatography is more flexible than either high-performance liquid chromatography or gas chromatography, as the density of the mobile phase can be modified during the course of the analysis. This feature can be exploited to increase the solvent strength of the supercritical fluid, thereby improving the solubility of the analytes. This can be achieved, for example, by creating gradients of pressure or temperature or by modifying the composition of the mobile phase; the latter option is particularly interesting as it also allows both the eluting power of the fluid and its selectivity to be modified.

The system used with a mobile phase of pure CO_2 gave perfect resolution of all nine compounds studied, but was slow (72 min). Generally, the addition of a polar solvent to supercritical CO_2 is used in order to obtain a shorter analysis time, but the selectivity is lower. Therefore, we tested the addition of an apolar solvent (hexane, cyclohexane, heptane and toluene) to CO_2. Among the solvents tested, cyclohexane gave the best selectivity for the three pairs of compounds which are notoriously difficult to separate: fluoranthene-pyrene, benzo(k)fluoranthene-benzo(b)fluoranthene, and indeno(1,2,3-c,d)pyrene-benzo(ghi)perylene.

Adding a gradient of cyclohexane to supercritical carbon dioxide decreases the analysis time because the density and solvent power of the mobile phase is greater than those for pure CO_2 (Table 1 and Fig. 2).

A multi-step gradient of cyclohexane (from 2 to 12%) in CO_2 at various temperatures (between 35 and 55 °C) and pressures (between 9 and 16 MPa) was tested. The best condition is a mobile phase of 100% CO_2 from 0 to 16 min, 96% CO_2 + 4% cyclohexane from 17 to 21 min, and 90% CO_2 + 10% cyclohexane from 22 to 29 min; a flow rate of 4.0 mL/min; 12 MPa pressure; and an oven temperature at 40 °C from 0 to 20 min, and 45 °C from 21 to 29 min.

The temperature program was required in order to improve the resolution of the indeno(1,2,3-c,d)pyrene-benzo(ghi)perylene pair. This difference slightly increased the analysis time. Nevertheless, the run time was 28 min.

Table 1. Pseudo-critical parameters and solubility parameter, d_m, of the CO_2-cyclohexane binary system. P_c' = pseudo-critical pressure, T_c' = pseudo-critical temperature, ρ'= density at 12 MPa and 40 °C, and ρ_l = density of the liquid mixture.

Cyclohexane	P_c'	T_c'	ρ'	$\rho_l = \rho_{co_2} + \rho$	$\delta_m = 1.25 \sqrt{P'_c} \frac{\rho'}{\rho_l}$
%	MPa	°C	g mL^{-1}	g mL^{-1}	cal cm^{-1}
0.00	7.380	31.10	0.728	1.232	6.35
2.00	7.291	33.77	0.756	1.220	6.61
4.00	7.203	36.49	0.778	1.210	6.82
5.00	7.160	37.87	0.787	1.206	6.90
8.00	7.032	42.09	0.809	1.195	7.12
10.00	6.948	44.98	0.820	1.183	7.25
12.00	6.866	47.92	0.829	1.167	7.35

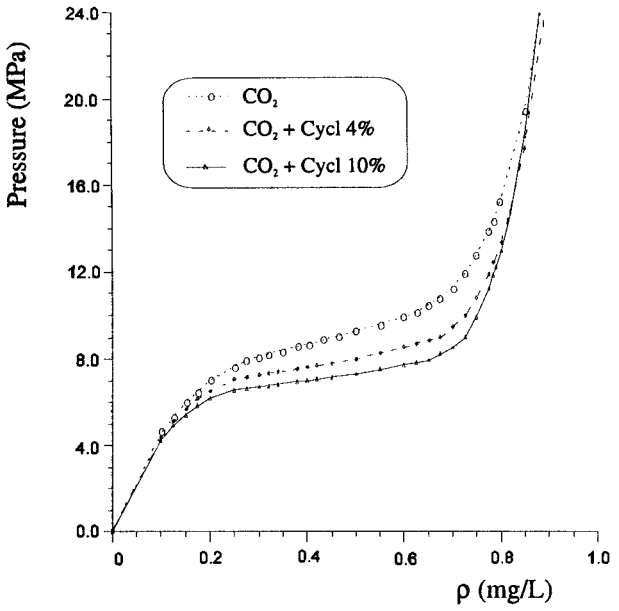

Figure 2. Estimated density of: CO_2, CO_2 + 4% cyclohexane at 40 °C, and CO_2 + 10% cyclohexane at 45 °C.

Sensitivity was improved by adding an injection system (Fig. 1) which eliminated sample solvent, permitting the amount injected to be increased by using the multiple application method without loss of resolution. Several conditions were tested in order to compare the peak areas and the selectivity of two pairs of compounds: fluoranthene-pyrene, and benzo(k)fluoranthene-benzo(b)fluoranthene (Table 2). The optimum injection accumulation conditions comprised a helium flow rate of 20.0 mL/min, an injection volume of 30.0 μL, and six injections.

The PAH mixture used in this study was representative of potentially carcinogenic hydrocarbons, according to French norms. The resulting chromatogram (Fig. 3) shows good resolution in a relatively short time (28 min). The baseline drift corresponds to the multi-step gradient. The compounds eluted in ascending order of aromatic nuclei. The selectivity obtained with the fluoranthene-pyrene pair and the indeno(1,2,3-cd)pyrene-benzo(ghi)perylene pair was very good. The linear regression equations and detection limits for each PAH are shown in Table 3. Interassay reproducibility is shown in Table 4; relative standard deviations were less than 7%.

In order to validate the technique on real world samples the concentrations of PAH were determined in Paris air samples (a) and in samples of vehicle emissions (b). Air samples were collected over several days. PAH were extracted (according to French norm X 43-025), combined and concentrated. Each exhaust sample comprised four soot extracts. PAH were identified by their retention times and using added standards. The results shown in Table V have the same magnitudes found by other laboratories

Table 2. Optimization of injection conditions (α_1=selectivity of fluoranthene/pyrene, and α_2 = selectivity of benzo(k)-fluoranthene / benzo(b)fluoranthene).

Injection volume: V_i (f_{He}=10 mL min^{-1})	Fluoranthene area / mV	Pyrene area / mV	α_1	α_2
20.0 μL	22441	22082	0.608	1.243
30.0 μL	24844	25596	0.620	1.290
40.0 μL	36643	36951	0.603	1.220
Helium flow : f_{He} (V_i = 30.0 μL)				
15 mL min^{-1}	26447	26292	0.619	1.350
20 mL min^{-1}	28018	28859	0.613	1.320
30 mL min^{-1}	27529	28718	0.609	1.270
Multiple application (f_{He}=20 mL min^{-1})				
2 x 30.0 μL	41074	44587	0.605	1.290
3 x 30.0 μL	81280	83114	0.607	1.320
4 x 30.0 μL	102350	106836	0.609	1.311
5 x 30.0 μL	133157	136643	0.604	1.308
6 x 30.0 μL	149872	153310	0.606	1.311
7 x 30.0 μL	150352	152989	0.602	1.307

Table 3. Linearity and detection limits (DL) under optimized conditions.

Code	Compound	Linear Regression Equation	r	DL/ng
F	Fluoranthene	Y = 0.142 + 3.31 X	0.996	15.8
P	Pyrene	Y = 0.171 + 3.23 X	0.995	17.8
B(a)A	Benz(a)anthracene	Y = -0.366 + 3.90 X	0.997	15.1
B(k)F	Benzo(k)fluoranthene	Y = 0.017 + 1.31 X	0.998	21.4
B(b)F	Benzo(b)fluoranthene	Y = -0.024 + 2.03 X	0.996	21.1
B(a)P	Benzo(a)pyrene	Y = -0.079 + 1.16 X	0.999	20.5
DB(ah)A	Dibenzo(a.h)anthracene	Y = -0.470 + 5.03 X	0.998	20.5
I(123cd)P	Indeno(1.2.3-c.d)Pyrene	Y = -0.254 + 1.42 X	0.997	24.8
B(ghi)P	Benzo(g.h.i)Perylene	Y = -0.198 + 3.14 X	0.996	25.4

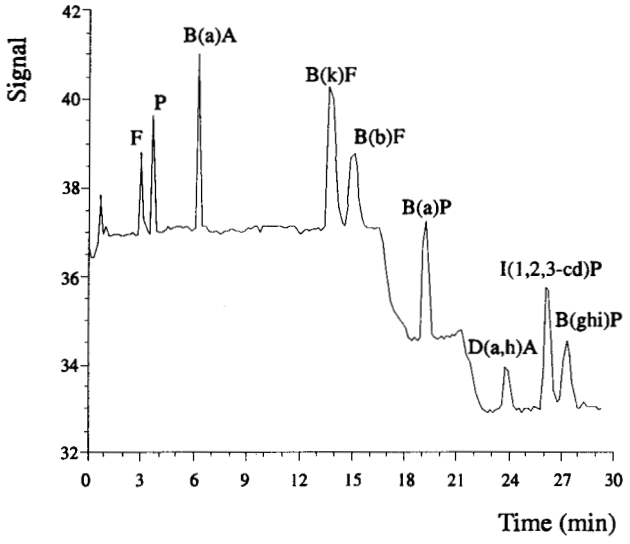

Figure 3. Chromatogram of a PAH mixture: Vydac C_{18} column; CO_2 mobile phase with an applied cyclohexane gradient of 4% from 17 to 21 min, and 10% from 22 to 29 min; pressure 12 MPa; temperature 40 °C from 0 to 20 min, and 45 °C from 21 to 29 min. Peak identifications in Table 3.

(Laboratoire d'Hygiène de la Ville de Paris and UTAC). The values in Tables 3, 4 and 5 and Fig. 3 indicate that this method can be applied to the analysis of PAH samples. Compared with HPLC[12] and GC[5] this method yields higher resolution. In addition, it is faster than HPLC methods.

Conclusion

The method proposed is linear (r = 0.992 to 0.998), reproducible (%S = 5.0 to 6.6), and gives good resolution for the analysis of PAH mixtures. The multi-step gradient of cyclohexane in CO_2 expands the scope of packed column SFC for the analysis of PAH. The injection method selected gives acceptable limits of detection; the development of spectrofluorimeters adapted to SFC promises to bring further improvements.

Table 4. Reproducibility of determinations under optimized conditions. Compound identification in Table 3.

Code	t_R/min	Area ± S	%S
F	3.23	14585 ± 794	5.4
P	3.87	25502 ± 1266	5.0
B(a)A	6.46	61520 ± 3048	5.0
B(k)F	14.02	99310 ± 5418	5.5
B(b)F	15.31	60156 ± 3084	5.1
B(a)P	19.58	65693 ± 4049	6.2
DB(ah)A	24.13	26017 ± 1487	5.7
I(123cd)P	26.47	87154 ± 5188	6.0
B(ghi)P	27.57	42230 ± 2795	6.6

Table 5. Results of the analysis of PAH from real samples. The concentrations refer to the extraction solutions. Chromatographic conditions in Fig. 3. Compound identifications in Table 3.

	(a) Paris Air Samples/mg L^{-1}	(b) Vehicle Exhaust Emissions/mg L-1
F	3.83	3.27
P	6.41	5.35
B(a)A	1.05	0.65
B(k)F	1.68	0.29
B(b)F	0.41	0.14
B(a)P	0.96	0.57
DB(ah)A	1.85	1.24
I(123cd)P	1.05	0.73
B(ghi)P	2.04	1.16

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