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Analysis of polycyclic aromatic hydrocarbons in cigarette samples using gel permeation chromatography clean-up by gas chromatography–tandem mass spectrometry

Wenliu Lian [a,b], Fenglian Ren [a,*], Liyun Tang [b], Daozhu Dong [b]

[a] Central South University, School of Chemistry and Chemical Engineering, No. 932 South Lushan Road, Changsha 410083, China
[b] China Tobacco Hunan Industrial Co., Ltd., Technology and Research Center, No. 386 Laodong Road, Changsha 410014, China

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ABSTRACT

A new method using gel permeation chromatography (GPC) as clean-up by gas chromatography coupled to a triple quadrupole tandem mass spectrometry (GC–MS/MS) was established to measure the concentrations of 16 polycyclic aromatic hydrocarbons (PAHs) in cigarette samples. GPC clean-up was employed to purify cigarette mainstream smoke samples collected from cigarettes in China, giving cleaner final extracts than traditional solid phase extraction (SPE). GC–MS/MS with a "pseudo" multiple reactive monitoring mode (PMRM) proved superior to the classic single quadrupole technique, with enhanced sensitivity and more accurate. Trace level PAHs could be readily confirmed by their retention times and characteristic ions. The concentrations of PAHs in these cigarette samples ranged from 455.9 ng/cig to 1201.3 ng/cig in the range of cigarette tar between 5 mg and 12 mg, with the main components being two-, three, and four-ring PAHs. When tar is over 10 mg, there is 14.4% increase in PAH concentrations on average than that tar is between 8 and 10 mg, while there is 28.6% decrease when tar is below 6 mg. These results indicate that relative low-tar cigarettes provide relative low emission levels of PAHs.

1. Introduction

Cigarette smoke is an aerosol stream containing an extremely complex mixture of chemicals [1], and more than 5000 constituents of smoke were identified, of which approximately 150 have been documented as toxicants [2–4].

Polycyclic aromatic hydrocarbons (PAHs), organic compounds containing two or more fused carboxylic aromatic rings, are highly stable contaminants generally found in trace amounts in the particulate matter of tobacco smoke in the presence of a very complex matrix [5,6]. PAHs are a very important group of chemical carcinogens, and 16 of these have been listed in the 93 "harmful and potentially harmful constituents" (HPHCs) for tobacco products by the US Food and Drug Administration (FDA) [7]. The analysis of PAHs in cigarette smoke usually involves solvent extraction [8] from total particulate matter (TPM), solid phase extraction [9–11], solid-phase microextraction [5], cleaning [12], and re-concentration [13] before analysis. These methods are complicated, time-consuming and laborious. Gel permeation chromatography (GPC) clean-up is a clear alternative for PAHs determination in complex matrix [14–16]. GPC can separate small and large molecules from interfering matrices, so it is easy to isolate contaminants from high molecular weight interferences. Chamberlain et al. [17] used GPC to separated oxygenated neutral constituents of cigarette smoke condensate from interfering phenolic compounds in 1979, few reports are available for GPC separation of cigarette smoke from then on.

The chromatographic detection of PAHs is usually carried out by liquid chromatography with fluorescence detection (HPLC–FLD) [18–23] or with ultraviolet-visible detector [24], or by gas chromatography with mass spectrometric detection (GC–MS) [25–30]. The former has a high sensitivity but presents an important disadvantage: the lack of unambiguous confirmation of the identity of the analytes. GC–MS avoids this disadvantage to some extent. The GC–MS technique has become established as the accepted method for PAHs determination in cigarette samples over 15 years [8]. Despite numerous improvements to single quadrupole MS instrumentation however, performance cannot match the sensitivity and specificity offered by triple quadrupole MS [31–36]. The very low concentration levels of PAHs set by the complex matrix nature of cigarette smoke has raised the need to develop simple, sensitive, selective, accurate analytical methods.

In this paper, we present a new method for the analysis of 16 PAHs in cigarette samples, based on a GPC clean-up step, followed by GC–MS/MS in pseudo MRM mode. The method was applied to determine PAH levels of cigarettes of different tar contents in China.

* Corresponding author.
E-mail address: fangxin78@163.com (F. Ren).

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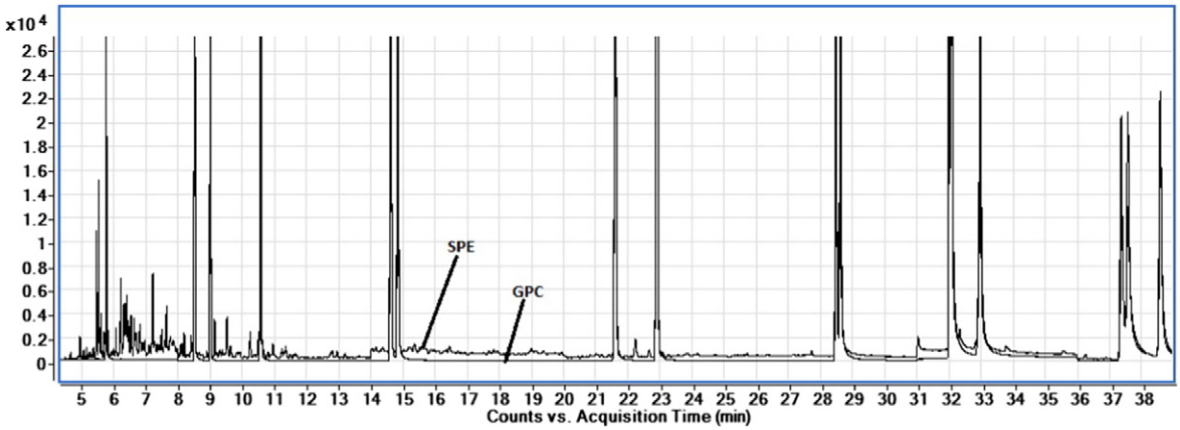

Fig. 1. Chromatograms for cigarette samples purified by GPC and SPE.

2. Materials and methods

2.1. Chemicals and materials

The isotope internal standard, naphthalene-d8, anthracene-d10, and benzo(a)pyrene-d12, were supplied from Dr. Ehrenstorfer GmbH (Augsburg, Germany); pesticide-quality solvents (cyclohexane, ethyl acetate) were supplied by honeywell (B&J, Muskegon, MI 49442, USA); PAH standard mix solutions at 2.0 mg/mL in cyclohexane were from Supelco (Bellefonte, PA, USA), and were stored in a freezer. Working standard solutions were prepared by appropriate dilution with ethyl acetate–cyclohexane (1:1, v/v) and stored under refrigeration at 4 °C.

According to EU commission regulation 333/2007, containers shall be rinsed with high purity acetone or hexane before use to minimize the risk of contamination. Wherever possible, apparatus and equipment coming into contact with the sample shall be made of inert materials such as aluminum, glass or polished stainless steel. Plastics such as polypropylene or PTFE shall be avoided because the analyte can absorb onto these materials [37].

2.2. Apparatus

Gas chromatography coupled to a triple quadrupole tandem mass spectrometer: GC–MS/MS analyses were carried out on an Agilent 7890A gas chromatograph and Agilent Technologies 7000 B triple quadrupole mass spectrometer equipped with a split/splitless injector and a model 7683 B autosampler (Agilent Technologies, Little Falls, DE, USA), fitted with an HP-5MS fused-silica capillary GC column (30 m × 0.25 mm I.D., 0.25 μm film thickness).

Gel permeation chromatograph: Vario GPC (LC Tech, German) consisted of a solvent delivery module, a fraction collector, and a GPC glass clean-up column (25 mm I.D. × 400 mm) packed with pre-swollen and washed Bio-Beads resin (Bio-Rad Labs., SX-3, 200–400 mesh) corresponding to 50 g of dry material, and a vacuum evaporator EVAIII (LC Tech, German) for extracts' concentration.

Model KQ-600E ultrasonic cleaning bath (Kunshan ultrasonic instrument company, PR China) was used for the ultrasonic extraction of PAHs in Cambridge pads which were used to collect the total particulate matter of cigarette mainstream smoke by a RM200 rotary 20 port smoking machine (Borgwaldt, German).

2.3. Sample pretreatment, extraction and purification

Cigarette samples were conditioned at a temperature of 22 ± 1 °C and 60 ± 2% relative humidity for a minimum of 48 h but not exceeding 10 days (ISO 3402:2000). Butt marking was to ISO butt length specifications. Filtered cigarettes were smoked to a measured butt length equaled to either the tipping paper +3 mm (ISO 4387:2000). All smoking was conducted in an environment of temperature 22 °C and 60% relative humidity. According to ISO 3308:2000, the smoking machine puffing parameters were 35 mL puff volume with 2.0 s puff duration once every 60.0 s. 20 cigarettes were smoked on the Borgwaldt rotary 20 port smoking machine. A 92 mm Cambridge pad was used to collect the total particulate matter of cigarette mainstream smoke.

Table 1
Pseudo multiple reactive monitoring mode (PMRM) and classic MRM transitions used for monitoring 16 PAHs in cigarette samples.

PAH	Retention time (min)	Quantitation PMRM transitions, m/z (collision energy (V))	Confirmation MRM transitions, m/z (collision energy (V))
Naphthalene (NAP)	5.78	128 > 128, (20)	128 > 102, (30)
Acenaphthylene (ANY)	8.58	152 > 152, (10)	152 > 151, (30)
Acenaphthene (ANA)	9.07	153 > 153, (5)	153 > 152, (40)
Fluorene (FLU)	10.65	166 > 166, (5)	166 > 165, (30)
Phenanthrene (PHE)	14.72	178 > 178, (5)	178 > 152, (30)
Anthracene (ANT)	14.94	178 > 178, (15)	178 > 152, (30)
Fluoranthene (FLT)	21.71	202 > 202, (10)	202 > 200, (40)
Pyrene (PYR)	22.92	202 > 202, (10)	202 > 200, (40)
Benzo(*a*)anthracene (BaA)	28.50	228 > 228, (15)	228 > 226, (40)
Chrysene (CHR)	28.64	228 > 228, (5)	228 > 226, (40)
Benzo[*b*]fluoranthene (BbF)	32.07	252 > 252, (20)	252 > 250, (40)
Benzo[*k*]fluoranthene (BkF)	32.10	252 > 252, (20)	252 > 250, (40)
Benzo[*a*]pyrene (BaP)	33.00	252 > 252, (20)	252 > 250, (40)
Indeno[1,2,3-*cd*]pyrene (IPY)	37.40	276 > 276, (20)	276 > 274, (50)
Dibenz(*a*,*h*)anthracene (DBA)	37.60	278 > 278, (5)	278 > 276, (50)
Benzo(*g*,*h*,*i*)perylene (BPE)	38.63	276 > 276, (5)	276 > 274, (50)