Quantitative Analysis of Polycyclic Aromatic Hydrocarbons (PAHs) Cited by the United States Food and Drug Administration *

by

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SUMMARY

The yields of 16 polycyclic aromatic hydrocarbons (PAHs) were determined from cigarette mainstream smoke condensate extracts using Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS). The method has been validated for ISO and Health Canada Intense (HCI) smoking protocols. Quantifiable levels (ISO means 0.16 to 365 ng/cig; HCI means 0.33 to 1595 ng/cig; $n = 30$) of 15 PAHs were found in the Kentucky reference cigarette K3R4F. The coefficient of variance (CV) was derived from ten determinations each run in triplicate. The CV range was 8.7% to 24.8% (ISO) and 6.6% to 24.3% (HCI). The limit of detection (LOD) based on empirical precision was ≤ 0.06 ng/cig (ISO) and ≤ 0.20 ng/cig (HCI) for all components except naphthalene (2.89 and 9.62 ng/cig, respectively). The yields from 5 unspecified branded cigarettes (Samples A-E) and 2 other reference cigarettes, K1R5F and the CORESTA monitor CM7, were determined under ISO smoking conditions. The same 15 PAHs were detected as in the K3R4F; however, cigarettes with lower yields of total particulate matter (TPM) were found to contain significantly less PAHs. One component was measured below the limit of quantification (LOQ) in Sample E and 2 components were $< \text{LOQ}$ in the K1R5F. [Beitr. Tabakforsch. Int. 25 (2013) 607–616]

ZUSAMMENFASSUNG

Die Ausbeuten von 16 polycyclischen aromatischen Kohlenwasserstoffen (PAK) wurden mittels Gaschromatographie/Tandem-Massenspektrometrie (GC-MS/MS) an

Extrakten vom Hauptstromrauchkondensat von Zigaretten bestimmt. Die Methode wurde nach den Rauchprotokollen von der ISO und Health Canada Intense (HCI) validiert. In der Kentucky-Referenzzigarette K3R4F wurden quantifizierbare Konzentrationen (nach ISO 0,16 bis 365 ng/Zigarette; nach HCI 0,33 bis 1595 ng/Zigarette; $n = 30$) von 15 PAK festgestellt. Der Variationskoeffizient (CV) wurde durch zehn Bestimmungen, jeweils dreifach durchgeführt, ermittelt. Der Bereich des CV betrug 8,7% bis 24,8% (ISO) bzw. 6,6% bis 24,3% (HCI). Die Nachweisgrenze basierend auf empirischer Genauigkeit betrug $\leq 0,06$ ng/Zigarette (ISO) bzw. $\leq 0,20$ ng/Zigarette (HCI) für alle Bestandteile mit Ausnahme von Naphthalen (2,89 bzw. 9,62 ng/Zigarette). Die Ausbeuten von 5 nicht spezifizierten Markenzigaretten (Proben A-E) und 2 weiteren Referenzzigaretten, K1R5F und der CORESTA Monitor CM7, wurden unter ISO-Rauchbedingungen bestimmt. Es wurden dieselben 15 PAK wie in der K3R4F festgestellt, jedoch enthielten Zigaretten mit geringeren Ausbeuten an Gesamtpartikelmasse (TPM) signifikant weniger PAK. Ein Bestandteil in Probe E und 2 Bestandteile in der K1R5F lagen unterhalb der Bestimmungsgrenze (LOQ). [Beitr. Tabakforsch. Int. 25 (2013) 607–616]

RESUME

Les rendements de 16 hydrocarbures aromatiques polycycliques (HAP) ont été déterminés à partir d'extraits de condensés du flux de fumée principal de cigarettes en utilisant la chromatographie en phase gazeuse-spectrométrie de masse en tandem (GC-MS/MS). La méthode a été validée pour les protocoles de fumage de la norme ISO et de Health

Canada Intense (HCI). Des niveaux quantifiables (soit pour ISO 0,16 à 365 ng/cig et pour HCI 0,33 à 1595 ng/cig; n = 30) de 15 HAP ont été trouvés dans la cigarette de référence K3R4F de type Kentucky. Le coefficient de variation (CV) a été dérivé de dix déterminations, chacune effectuée en triple exemplaire. L'intervalle du CV était de 8,7% à 24,8% (ISO) et de 6,6% à 24,3% (HCI). La limite de détection basée sur une précision empirique était $\leq 0,06$ ng/cig (ISO) et $\leq 0,20$ ng/cig (HCI) pour tous les composants, à l'exception du naphthalène (respectivement 2,89 et 9,62 ng/cig). Les rendements de 5 cigarettes de marques non spécifiées (échantillons A-E) et de 2 autres cigarettes de référence, K1R5F et CORESTA monitor CM7, ont été déterminés dans les conditions de fumage de la norme ISO. Les 15 HAP détectés étaient les mêmes que dans la cigarette K3R4F; cependant, des cigarettes avec des rendements plus faibles de particules en suspension totales (PST) se sont avérées contenir beaucoup moins de HAP. Un composant était inférieur au seuil de quantification (SDQ) dans l'échantillon E et 2 composants étaient $< \text{SDQ}$ dans l'échantillon K1R5F. [Beitr. Tabakforsch. Int. 25 (2013) 607–616]

LIST OF ABBREVIATIONS

5-MC	5-Methylchrysene
B[a]A	Benz[a]anthracene
B[a]P	Benzo[a]pyrene
B[c]P	Benzo[c]phenanthrene
B[b]F	Benzo[b]fluoranthene
B[k]F	Benzo[k]fluoranthene
B[j]A	Benz[j]aceanthrylene
C[cd]P	Cyclopenta[c,d]pyrene
CORESTA	Cooperation Centre for Scientific Research Relative to Tobacco
CV	Coefficient of Variance
dB[ah]A	Dibenz[a,h]anthracene
dB[al]P	Dibenzo[a,l]pyrene
dB[ae]P	Dibenzo[a,e]pyrene
dB[ai]P	Dibenzo[a,i]pyrene
dB[ah]P	Dibenzo[a,h]pyrene
FDA	Food and Drug Administration
GC-MS	Gas Chromatography-Mass Spectrometry
GC-MS/MS	Gas Chromatography-Tandem Mass Spectrometry
I[cd]P	Indeno[1,2,3-cd]pyrene
ISO	International Organisation for Standardisation
ISTD	Internal Standard
HCI	Health Canada Intense
HPHC	Harmful and Potentially Harmful Constituents
LOD	Limit of Detection
LOQ	Limit of Quantification
MRM	Multiple Reactions Monitoring
NFDPM	Nicotine-Free Dry Particulate Matter
PAH	Polycyclic Aromatic Hydrocarbons
SIM	Selective Ion Monitoring
TPM	Total Particulate Matter
TTI	Total Throughput Ion

INTRODUCTION

The carcinogenic properties of polycyclic aromatic hydrocarbons (PAHs) are well documented (1–7). Benzo[a]pyrene (B[a]P) was first isolated from coal 'tar' in the 1930s and its carcinogenicity demonstrated on the skin of mice (2). BLACKLOCK demonstrated its tumourigenic properties in the lungs of rats (3). It is one of the constituents of tobacco smoke identified and catalogued by WYNDER and HOFFMANN (4). It has been widely regarded as a standard marker of overall PAH exposure level in cigarette smoke (7). The testing of 44 so-called 'Hoffmann Analytes' is mandatory in Brazil and Canada and, since 2009, legislation passed in the United States, brought in new regulatory requirements for tobacco product brands marketed in the USA (8). The United States Food and Drug Administration (FDA) identified 93 harmful and potentially harmful constituents (HPHC) including 16 PAH compounds (Table 1). An abbreviated list containing 20 HPHC compounds including B[a]P was made compulsory for the industry in 2012 (9).

Table 1. PAH compounds defined by the FDA as harmful and potentially harmful constituents (HPHC)

Naphthalene *	
Benzo[c]phenanthrene	B[c]P
Benz[a]anthracene	B[a]A
Cyclopenta[c,d]pyrene	C[cd]P
Chrysene	
5-Methylchrysene	5-MC
Benzo[b]fluoranthene	B[b]F
Benzo[k]fluoranthene	B[k]F
Benz[j]aceanthrylene	B[j]A
Benzo[a]pyrene	B[a]P
Indeno[1,2,3-cd] pyrene	I[cd]P
Dibenz[a,h]anthracene	dB[ah]A
Dibenzo[a,l]pyrene	dB[al]P
Dibenzo[a,e]pyrene	dB[ae]P
Dibenzo[a,i]pyrene	dB[ai]P
Dibenzo[a,h]pyrene	dB[ah]P

* Strictly speaking naphthalene is a bicyclic aromatic hydrocarbon

PAHs consist of multiple aromatic rings formed through incomplete combustion of carbon fuel material such as tobacco (Figure 1). They are highly lipophilic and readily enter the biomass, being released back into the system by the process of lipolysis (10). They can be separated using high performance liquid chromatography (HPLC) and gas chromatography (GC), and analysed using fluorescence spectroscopy (HPLC-FLD) and mass spectrometry (LC-MS, GC-MS). GC-MS analysis has been adopted into the ISO standardised method for B[a]P in mainstream smoke [11]. Analysis of all of the remaining 15 PAHs on the FDA list provides certain challenges due to high matrix background in the smoke condensate extracts. Advances in triple quadrupole mass spectrometry have enabled detection limits of < 1 ppb in complex matrices to be realised (12). Removal of matrix interference across the triple-stage mass analyser and subsequent lowering of the baseline is the principle of the operation. Essentially it provides an added dimension of resolution compared to GC-MS. Ions are resolved in the first quadrupole (Q1) by their

Figure 1. Examples of 2-, 4-, 5-, and 6-ring PAHs:

(a) Naphthalene; (b) Benz[a]anthracene; (c) 5-Methylchrysene; (d) Benzo[a]pyrene; (e) Dibenzo[a,e]pyrene.
Figures obtained from Chemspider, free chemical database provided by the Royal Society of Chemistry

mass to charge (m/z) in the same way as single ion monitoring (SIM) would be used in a single-stage quadrupole. A collision cell (Q2) is placed after Q1. This is actually a non-scanning hexapole held at total throughput ion (TTI). By application of a collision gas (nitrogen or argon) and a collision voltage (C_c), further fragmentation of the ions occurs which are then resolved in the second quadrupole (Q3). This provides the system with its unique transition spectra known as multiple reaction monitoring (MRM).

Data for B[a]P yields in reference cigarettes are well established and have been generated from various inter-laboratory collaborations (13–16). Data has been gathered from laboratories using GC-MS and HPLC-FLD. The Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) recommended method for B[a]P involves GC-MS (SIM) analysis (13). This is the standardised method ISO 22634 (11). Standardised smoking conditions are defined in ISO 3308 (17). Other smoking protocols such as the Health Canada Intense (HCI) (18) aim to more closely reflect potential maximum levels. A collaborative study conducted by CORESTA, in 2012, involving 16 laboratories, reported mean yield, repeatability (r) and reproducibility (R) data from 10 cigarette brands including two University of Kentucky reference cigarettes, K3R4F and K1R5F, and the CORESTA monitor CM6 under ISO and HCI protocols. B[a]P levels generally correlate well with ‘tar’ levels or more specifically the nicotine-free dry particulate matter (NFDPM): K3R4F (NFDPM 8 mg/cig; ISO mean 6.66 ng/cig, $R = 2.00$; HCI mean 15.51 ng/cig, $R = 4.93$), K1R5F (NFDPM 2 mg/cig; ISO mean 1.64 ng/cig, $R = 0.80$; HCI mean 7.13 ng/cig, $R = 2.28$), and CM6 (NFDPM 15 mg/cig; ISO mean 15.48 ng/cig, $R = 5.19$; HCI mean 27.86 ng/cig, $R = 8.45$) (13).

Various studies have been conducted to assess PAH yields in cigarettes (19–23). Yield data from reference cigarettes is limited, however, studies of the K1R4F (NFDPM 8 mg/cig) have been reported for some PAHs on the FDA list (20–22). RODGMAN and GREEN reported ISO values for naphthalene (342 ng/cig), benz[a]anthracene (B[a]A) (12.4 ng/cig), benzo[b]fluoranthene (B[b]F) (5.5 ng/cig), benzo[k]fluoranthene (B[k]F) (1.3 ng/cig), B[a]P (5.7 ng/cig), chrysene (13.6 ng/cig), dibenz[a,h]anthracene (dB[ah]A) (0.4 ng/cig), indeno[1,2,3-*cd*]pyrene (I[cd]P) (3.5 ng/cig), and naphthalene (342 ng/cig) (22). ROEMER *et al.* reported PAH ISO values for the K2R4F which super-

seded the K1R4F (23). The same group also reported ISO and HCI values for the next iteration K3R4F: B[a]A (ISO 11.8 ng/cig, HCI 29.8 ng/cig), B[b]F (ISO 5.09 ng/cig; HCI 13.2 ng/cig), B[k]F (ISO 2.02 ng/cig; HCI 5.38 ng/cig), B[a]P (ISO 6.73 ng/cig; HCI 16.2 ng/cig), I[cd]P (ISO 2.87 ng/cig; HCI 7.37 ng/cig), and dibenzo[a,e]pyrene dB[ae]P (ISO 0.173 ng/cig; HCI 0.858 ng/cig). Other PAHs, dB[ah]A, dibenzo[a,h]pyrene (dB[ah]P), dibenzo[a,i]pyrene (dB[ai]P), dibenzo[a,l]pyrene (dB[al]P) and 5-methylchrysene (5-MC), were reported below LOQ under both regimes.

Validated data for the determination of 16 PAH compounds using an adapted version of ISO 22634, under ISO and HCI smoking regimes are presented in this manuscript.

EXPERIMENTAL

Materials and equipment

The Kentucky reference cigarettes were supplied by the Kentucky Tobacco Research and Development Center (Lexington, KY, USA). CORESTA monitor cigarettes were supplied by Cerulean (Milton Keynes, UK). Branded cigarettes were purchased in the United States. The cigarettes were smoked on a 20-port rotary smoking machine (RM20/CS or RM20D, Borgwaldt, Hamburg, Germany) compliant with ISO 3308 (17). Linear smoking was performed using a Cerulean SM450. Materials used for smoking and extraction are outlined in ISO 22634 (11). Cambridge filter (CF) glass fibre pads were manufactured by Whatman (Maidstone, UK) and supplied by Cerulean. Solid-phase extraction (SPE) CH(EC) cartridges (1 g, 10 mL) were supplied by Biotage (Cardiff, UK). TurboVap was supplied by Biotage. Analyte stock standards were supplied by Accustandard (New Haven, CT, USA) and Toronto Research Chemicals (Toronto, Canada). Deuterated PAH analogues used as internal standards were supplied by Cambridge Isotope Laboratories (Andover, MA, USA). The analysis was performed using GC-MS/MS (7890GC-7000MS/MS, Agilent, Santa Clara, CA, USA). Components were separated using a J&W PAH SELECT (30m $\times$ 0.25 mm, 0.15 mm) column (Agilent). Peak integration and processing was performed using Mass Hunter software (Agilent). Gases used were helium and nitrogen (> 99.999%).

Sample preparation

All cigarettes were conditioned in accordance with ISO 3402 (24). NFDPM values were determined using a linear smoking machine in accordance with ISO 4387 (25). Smoking was carried out in accordance with ISO 3308 (17) and HCI (18) protocols. Smoking parameters are outlined in Table 2. For HCI smoking all filter ventilation holes were blocked. Smoking for PAHs was performed using a rotary smoking machine. The smoke condensate from 20 cigarettes (ISO) and 10 cigarettes (HCI) was trapped onto a 92 mm CF pad. TPM values were obtained for all of the samples in accordance with ISO 4387 (25). The extraction and clean-up procedure is outlined in ISO 22634 (11). The CF pad was spiked with internal standard solution then extracted into 50 mL methanol, shaken to disintegration and filtered through a Buchner funnel. The filtrate was diluted with water to approximately 60% then the clean-up was performed using CH(EC) cartridge. PAHs were eluted with 15 mL cyclohexane prior to concentration to 1 mL using a TurboVap.

Table 2. ISO and HCI smoking regimes

Smoking regime	Puff volume (mL)	Puff frequency (s)	Puff duration (s)	Ventilation blocking (%)
ISO 3308:2009	35	60	2	0
HCI T-115	55	30	2	100

GC-MS/MS

The inlet was held at 300 °C (1 mL; splitless 1 min). The carrier gas was helium (1.2 mL/min). Oven start temperature was 60 °C (1 min) then ramped at 25 °C/min to 150 °C (0.5 min) then 3 °C/min to 325 °C (12 min). Transfer line was held at 310 °C. The ion source was held at 300 °C (EI; 70eV). Helium quench gas and nitrogen were supplied to the collision cell (2.3 and 1.5 mL/min, respectively).

Table 3. Analytes and deuterated internal standards

Analyte	Internal standard
Naphthalene	Naphthalene D8
Benzo[c]phenanthrene	Benzo[a]anthracene D12
Benzo[a]anthracene	Benzo[a]anthracene D12
Cyclopenta[c,d]pyrene	Benzo[a]anthracene D12
Chrysene	Chrysene D12
5-Methylchrysene	5-Methylchrysene D3
Benzo[b]fluoranthene	Benzo[b]fluoranthene D12
Benzo[k]fluoranthene	Benzo[k]fluoranthene D12
Benz[<i>l</i>]aceanthrylene	B[a]P D12
B[a]P	B[a]P D12
Indeno[1,2,3- <i>cd</i>]pyrene	Indeno[1,2,3- <i>cd</i>]pyrene D12
Dibenzo[a,h]anthracene	Dibenzo[a,h]anthracene D14
Dibenzo[a,i]pyrene	Dibenzo[a,i]pyrene D14
Dibenzo[a,e]pyrene	Dibenzo[a,i]pyrene D14
Dibenzo[a,j]pyrene	Dibenzo[a,i]pyrene D14
Dibenzo[a,h]pyrene	Dibenzo[a,i]pyrene D14

Calibration and internal standards

Standards were prepared in hexane of 16 PAH analytes in a concentration range of 2 to 200 ng/mL (except B[a]A, chrysene 4 to 400 ng/mL and naphthalene 200 to 5000 ng/mL). Deuterated analogues of 10 components were added as internal standards (ISTDs) and spiked onto the Cambridge filter pads prior to addition of extraction solvent. Six compounds were quantified using surrogate internal standards (Table 3).

Limit of detection/Limit of quantification

Ten analyses of the lowest calibration standard were ran on 10 separate sequence runs. Calculated values within $\pm 15\%$ of the nominal value were obtained and the LOD was defined as $3 \times$ standard deviation (SD) and the LOQ defined as $10 \times$ SD. The units were converted into nanograms per cigarette (ng/cig).

K3R4F analysis

PAHs were determined for the K3R4F under ISO and HCI smoking regimes. Analyses were performed in triplicate and 10 determinations made for each regime on separate days. The overall mean and standard error were calculated from the individual mean value from each determination. The intraday precision was calculated from the individual SD value from each determination.

The relative recoveries were determined by pad spiking K3R4F extracts: calibration level 2 (CL2) 5:10:500 ng/mL; CL3 10:20:1000 ng/mL; and CL4 20:40:2000 ng/mL, prior to addition of extraction solvent. A pad spike at each level and an unspiked monitor were run in triplicate.

Branded and other reference cigarettes

Five branded cigarettes and two other reference cigarettes (CM7 and K1R5F) were analysed under ISO smoking conditions. All branded cigarettes were ventilated. Brands A to C were standard circumference, Brands D and E were Slim type cigarettes. One analysis was performed in triplicate. A K3R4F determination was also run as a control.

RESULTS AND DISCUSSION

K3R4F yield data

The PAH yields along with the standard errors are shown in Table 4. There were 15 quantifiable compounds. Only dB[a]P was determined to be below LOD. B[a]P levels were consistent with published data and our own data using the GC-MS method. Naphthalene was by far the most abundant (ISO mean 365 ng/cig and HCI mean 1595 ng/cig) but also quite variable under ISO conditions (CV 20.5%). The remaining 14 PAHs were present between 0.16 and 13.95 ng/cig (ISO). The most variable data, dB[ai]P and dB[ah]P (CV 24.3–24.8% ISO), were also detected at the lowest levels (0.16–0.17 ng/cig ISO). This was close to the LOQs for these compounds. Generally mean yields were more variable under ISO conditions.

Table 4. PAH mean yields in the K3R4F under ISO and HCl regimes

PAH ISO and HCl mean yields	K3R4F ISO (TPM 10.0 mg/cig; n = 30)		K3R4F HCl (TPM 40.4 mg/cig; n = 30)	
	ng/cig	SE	ng/cig	SE
Naphthalene	365.20	74.40	1594.80	153.00
Benzo[c]phenanthrene	1.19	0.16	3.05	0.20
Benz[a]anthracene	10.90	1.13	28.17	1.70
Cyclopenta[c,d]pyrene	6.75	0.59	17.69	1.21
Chrysene	13.95	1.67	32.39	3.76
5-Methylchrysene	1.09	0.21	2.73	0.55
Benzo[b]fluoranthene	4.65	0.60	12.81	0.98
Benzo[k]fluoranthene	2.03	0.29	5.05	0.37
Benz[j]aceanthrylene	1.48	0.27	3.69	0.69
B[a]P	6.07	0.79	15.64	1.29
Indeno[1,2,3-cd]pyrene	2.72	0.37	6.81	0.58
Dibenzo[a,h]anthracene	0.66	0.10	1.73	0.15
Dibenzo[a,i]pyrene	< LOD	N/A	< LOD	N/A
Dibenzo[a,e]pyrene	0.24	0.03	0.62	0.12
Dibenzo[a,f]pyrene	0.17	0.04	0.39	0.10
Dibenzo[a,h]pyrene	0.16	0.04	0.33	0.07

This may reflect variability of the TPM (CV 7.0% ISO, 3.4% HCl). Different rotary smoking machines were used and although strict tolerance limits on the TPM were employed it was observed that small differences in TPM greatly affected overall PAH yields. Yields under HCl conditions were generally 2 to 3 times higher compared with ISO. The higher yields, hence more signal, may explain the greater precision observed. No significant difference was observed in the overall precision between PAHs with deuterated analogue ISTDs and those with surrogate ISTDs. The intraday precision was also highest for the lowest yielding PAHs (Table 5).

Chromatography and mass spectral analysis

Positive identification was based on a retention time (RT) match (± 1 s) of the peak apex of the sample against the peak apex of a calibration check standard. PAHs were resolved by multiple reaction monitoring (MRM) or tandem selected ion monitoring (t-SIM) (Table 6). The dwell and C_v were optimised using product ion scan. Peaks are shown to be clearly resolved from the matrix (Figure 2). Background noise and matrix interference were found to be minimal compared with GC-MS. The SELECT PAH column is a moderately polar phase type, therefore, separation was by volatility and polarity. Structural isomers were clearly separated.

Limit of detection/quantification

Repeat analyses of the lowest calibration standard were used to calculate LOD/LOQ (Table 7). It is an empirical measurement based on a procedure outlined in the Health Canada official method for the determination of B[a]P in mainstream cigarette smoke (26). The selectivity of the method and detection of certain PAHs at very low levels do suggest that these threshold levels are achievable despite the matrix present.

Table 5. Intraday precision derived from the average SD from the K3R4F data, expressed as a percentage (CV.r (%)), under ISO and HCl regimes

Analyte	CV.r (%) ISO	CV.r (%) HCl
Naphthalene	6.3	5.1
Benzo[c]phenanthrene	4.6	4.3
Benz[a]anthracene	3.9	3.1
Cyclopenta[c,d]pyrene	3.9	4.1
Chrysene	4.3	3.1
5-Methylchrysene	5.1	5.9
Benzo[b]fluoranthene	2.8	3.2
Benzo[k]fluoranthene	4.7	3.9
Benz[j]aceanthrylene	8.2	5.6
B[a]P	3.2	3.2
Indeno[1,2,3-cd]pyrene	3.1	3.3
Dibenzo[a,h]anthracene	4.3	5.0
Dibenzo[a,i]pyrene	N/A	N/A
Dibenzo[a,e]pyrene	12.3	8.6
Dibenzo[a,f]pyrene	19.1	11.9
Dibenzo[a,h]pyrene	19.4	14.8

Branded cigarettes and other monitor data

A comparison of PAH yields between 5 branded cigarettes and 3 reference cigarettes is shown in Table 8. In all products only dB[a]P was determined to be below LOD. The CM7 was the only non-ventilated filter product with the highest TPM (15.3 mg/cig) and produced the highest PAH yields. The K1R5F produced the lowest with 2 components determined < LOQ. Sample A contained the highest PAH yields of the branded products. Sample B was the highest yielding TPM (14.6 mg/cig) product and contained the second highest PAH yields. However, 'tar' yields obtained from linear smoking under ISO 4387 (25) found NFDPM in Samples A and B to be closer than the TPM data from rotary smoking (Table 9).

Table 6. Transition ions (m/z) and analyser settings

Analyte	MRM quantifier (qualifier)	Collision energy (V)	Dwell (ms)	RT (min)
Naphthalene D8	136.1–108.1	25	10	5.14
Naphthalene	128.1–102.1 (128.1–78.0)	20 (25)	15 (15)	5.16
Benzo[c]phenanthrene	228.0–226.1	40	115	17.57
Benz[a]anthracene D12	240.0–236.1	40	36	18.57
Benz[a]anthracene	228.0–228.1	20	100	18.72
Cyclopenta[c,d]pyrene	225.9–224.1	40	100	19.07
Chrysene D12	240.0–236.1	40	36	19.00
Chrysene	228.0–228.1	20	100	19.18
5-Methylchrysene D3	245.0–244.1	20	25	21.70
5-Methylchrysene	242.0–241.1(242.0–226.1)	20 (35)	67 (67)	21.78
Benzo[b]fluoranthene D12	264.0–264.2	20	20	25.50
Benzo[b]fluoranthene	252.0–252.1(252.0–250.1)	20 (35)	100 (100)	25.69
Benzo[k]fluoranthene D12	264.0–264.2	20	20	25.59
Benzo[k]fluoranthene	252.0–252.1(252.0–250.1)	20 (35)	100 (100)	25.84
Benz[j]aceanthrylene	252.0–252.1(252.0–250.1)	20 (45)	110 (110)	26.70
B[a]P D12	264.2–260.2	45	30	28.11
B[a]P	252.0–252.1(252.0–250.1)	20 (45)	110 (110)	28.29
Indeno[1,2,3-cd]pyrene D12	288.0–288.2	30	30	34.85
Indeno[1,2,3-cd]pyrene	276.0–276.1	30	80	35.03
Dibenzo[a,h]anthracene D14	292.0–288.3	40	30	34.86
Dibenzo[a,h]anthracene	278.0–278.1	30	125	35.07
Dibenzo[a,i]pyrene D14	316.0–316.2	20	50	46.82
Dibenzo[a,i]pyrene	302.0–302.1(302.0–300.1)	20 (40)	160 (100)	43.30
Dibenzo[a,e]pyrene	302.0–302.1(302.0–300.1)	20 (40)	160 (100)	45.71
Dibenzo[a,i]pyrene	302.0–302.1(302.0–300.1)	20 (40)	160 (100)	47.15
Dibenzo[a,h]pyrene	302.0–302.1(302.0–300.1)	20 (40)	160 (100)	47.92

Table 7. Limits of detection/quantification (LOD/LOQ)

Analyte	ISO LOD (ng/cig)	ISO LOQ (ng/cig)	HCI LOD (ng/cig)	HCO LOQ (ng/cig)
Naphthalene	2.89	9.62	5.77	19.24
Benzo[c]phenanthrene	0.02	0.08	0.05	0.15
Benz[a]anthracene	0.06	0.20	0.12	0.39
Cyclopenta[c,d]pyrene	0.03	0.09	0.05	0.17
Chrysene	0.04	0.12	0.07	0.25
5-Methylchrysene	0.02	0.05	0.03	0.11
Benzo[b]fluoranthene	0.02	0.08	0.05	0.15
Benzo[k]fluoranthene	0.02	0.06	0.04	0.13
Benz[j]aceanthrylene	0.05	0.16	0.10	0.33
B[a]P	0.01	0.04	0.02	0.08
Indeno[1,2,3-cd] pyrene	0.02	0.08	0.05	0.17
Dibenzo[a,h]anthracene	0.02	0.08	0.05	0.16
Dibenzo[a,i]pyrene	0.04	0.13	0.08	0.25
Dibenzo[a,e]pyrene	0.03	0.10	0.06	0.20
Dibenzo[a,i]pyrene	0.02	0.07	0.04	0.14
Dibenzo[a,h]pyrene	0.03	0.11	0.07	0.23

Figure 2. Selected chromatograms from K3R4F extracts:
(a) Naphthalene; (b) 5-MC; (c) B[b]F; (d) B[a]P; (e) dB[ah]A; (f) dB[ae]P

Table 8. Comparison of PAH yields of branded and reference cigarettes

Sample	K3R4F	K1R5F	CM7	Sample A	Sample B	Sample C	Sample D slim	Sample E slim
TPM (mg/cig; n = 3)	9.8	2.3	15.3	11.9	14.6	10.0	9.7	6.8
PAH ISO mean yields	ng/cig	ng/cig	ng/cig	ng/cig	ng/cig	ng/cig	ng/cig	ng/cig
Naphthalene	461.1	192.3	1542.7	606.0	508.5	578.9	554.0	415.4
Benzo[c]phenanthrene	1.28	0.44	3.26	2.29	1.85	1.33	0.99	0.78
Benz[a]anthracene	11.48	3.05	24.71	17.48	14.78	11.30	7.66	5.84
Cyclopenta[c,d]pyrene	7.38	2.37	21.28	13.53	10.77	7.77	5.21	3.74
Chrysene	16.42	4.75	41.06	24.07	20.34	16.03	10.76	8.98
5-Methylchrysene	1.24	0.31	2.20	1.60	1.55	1.15	0.81	0.62
Benzo[b]fluoranthene	5.37	1.99	16.72	8.81	7.46	5.55	3.71	2.75
Benzo[k]fluoranthene	2.26	0.77	8.06	4.24	3.38	2.29	1.50	1.15
Benz[j]aceanthrylene	1.50	0.65	4.53	2.14	2.15	1.60	1.19	0.89
B[a]P	6.88	2.00	18.39	10.81	9.11	6.69	4.38	3.37
Indeno[1,2,3-cd] pyrene	3.01	0.93	8.45	4.75	4.12	2.99	1.98	1.52
Dibenz[a,h]anthracene	0.72	0.23	1.78	0.99	0.94	0.70	0.48	0.35
Dibenzo[a,i]pyrene	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Dibenzo[a,e]pyrene	0.30	0.10	0.67	0.39	0.36	0.24	0.18	0.13
Dibenzo[a,i]pyrene	0.18	< 0.07	0.36	0.22	0.20	0.12	0.08	0.07
Dibenzo[a,h]pyrene	0.19	< 0.11	0.29	0.21	0.20	0.13	< 0.11	< 0.11

Table 9. Comparison of ‘tar’ yields from rotary and linear machine smoking

Product	Rotary ISO 4387	Linear ISO 4387	
	TPM (mg/cig)	TPM (mg/cig)	NFDPM (mg/cig)
K3R4F	9.8	9.8	8.5
K1R5F	2.3	2.2	1.9
CM7	15.3	16.5	13.7
Sample A	11.9	11.9	9.8
Sample B	14.6	13.4	11.1
Sample C	10.0	10.6	8.9
Sample D	9.7	9.8	8.6
Sample E	6.8	7.5	6.5

Slim type samples D and E produced the lowest PAH yields; dB[ah]P was < LOQ in these products. ‘Tar’ level was shown to be the main driver of PAH yields but type of ventilation, tobacco blend and circumference must also be factors to consider. American type blends have been found to yield less B[a]P per unit of ‘tar’ than Virginia blends (27). Yields of B[a]P proportional to the other PAHs was widely consistent across all of the products. The exception was naphthalene which may reflect the variance found in the K3R4F validation data.

Recoveries

The relative recoveries were calculated from the percentage ratio of the actual versus theoretical mean from 3 spiked and 3 unspiked K3R4F replicates at 3 CLs (Table 10). Recoveries were acceptable in all cases and ranged from 83.4% to 124.2%. All the recoveries except three were within 15% of the nominal values. Greater accuracy was observed using analogue ISTDs (86.7% to 107.7%) compared with surrogates ISTDs (83.4 % to 124.2 %). Pads spiked at CL2 were equivalent to addition of

Table 10. Relative recoveries obtained at 3 calibration levels

	CL2	CL3	CL4
Naphthalene	105.5%	105.4%	107.7%
Benzo[c]phenanthrene	90.7%	80.8%	85.2%
Benzo[a]anthracene	98.4%	92.8%	91.5%
Cyclopenta[c,d]pyrene	112.2%	111.0%	124.2%
Chrysene	103.2%	102.2%	101.7%
5-Methylchrysene	98.6%	93.7%	86.7%
Benzo[b]fluoranthene	103.6%	98.4%	95.2%
Benzo[k]fluoranthene	98.3%	104.7%	102.9%
B[a]P	101.0%	99.8%	97.0%
Indeno[1,2,3-cd] pyrene	98.1%	90.4%	96.3%
Dibenz[a,h]anthracene	101.9%	97.5%	96.0%
Dibenzo[a,i]pyrene	93.0%	111.9%	99.6%
Dibenzo[a,e]pyrene	84.5%	112.7%	83.4%
Dibenzo[a,i]pyrene	91.8%	94.8%	88.0%
Dibenzo[a,h]pyrene	97.2%	87.8%	105.6%

0.25 ng/cig (ISO) except B[a]A, chrysene (0.50 ng/cig) and naphthalene (25 ng/cig).

CONCLUSIONS

Yields are consistent with previous studies (19–23). The least abundant PAHs are those with the most rings. This study was able to measure three out of the four 6-ring PAHs at sub-ng/cig levels in the K3R4F. Some 4- and 5-ring PAHs were also determined at sub-ng/cig in lower ‘tar’ products. The matrix is minimalized to such an extent that these detection levels are comfortably attainable. Only one component, dB[a]P, was not detected which was due to matrix interference rather than lack of sensitivity. A retention time match (± 1 s) was not achievable for this compound. However, the recovery for this compound in

the lowest spiked extract (CL2; 0.25 ng/cig) was 93%. This method is only concerned with the particulate phase of the smoke aerosol, however, naphthalene has a relatively high vapour pressure (11.6 Pa; 25 °C) making it a semi-volatile compound which will partition between the vapour- and particulate- phases. Therefore, levels of naphthalene in whole smoke would be expected to be higher. The other PAHs on the FDA list have much lower relative vapour pressures ($< 2.8 \times 10^{-5}$ Pa; 25 °C) and are found almost entirely in the particulate phase (21, 28).

We have demonstrated that FDA PAHs can be measured in the smoke condensate using an adapted version of ISO 22634: Determination of B[a]P in Cigarette Mainstream Smoke, allowing for continuity from existing regulatory requirements. B[a]P is currently the standard marker for overall PAH exposure from tobacco smoke. This method provides the flexibility to be able to determine B[a]P and other PAHs if required. Levels of 6-ring and some 4- and 5-ring PAHs were found to be very low; therefore, triple-stage quadrupole MS provides a reliable means to see these components without employing complex clean-up procedures.

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