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RESEARCH PAPER

QUANTITATIVE ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) CITED BY THE UNITED STATES FOOD AND DRUG ADMINISTRATION

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Summary

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 [1]. The United States Food and Drug Administration (FDA) identified 93 harmful and potentially harmful constituents (HPHC) including 16 polycyclic aromatic hydrocarbons (PAHs) (Table 1). An abbreviated list containing 20 HPHC compounds including benzo[a]pyrene (B[a]P) was made compulsory for the industry in 2012 [2].

The yields of 16 PAHs were determined from cigarette mainstream smoke (MSS) 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. The yields of Kentucky reference cigarettes (K3R4F and K1R5F), CORESTA monitor cigarette (CM7), and 5 commercially available cigarettes were determined.

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

Naphthalene
Benzo[c]phenanthrene
Benz[a]anthracene
Cyclopenta[c,d]pyrene
Chrysene
5-Methylchrysene
Benzo[b]fluoranthene
Benzo[k]fluoranthene
Benz[j]aceanthrylene
Benzo[a]pyrene
Indeno[1,2,3-cd] pyrene
Dibenz[a,h]anthracene dB[ah]P
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

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are formed through incomplete combustion of carbon fuel material such as tobacco. Analysis provides certain challenges due to high matrix background in the smoke condensate extracts. Advances in triple quadrupole mass spectrometry have enabled detection of these compounds in complex matrices to be realised.

Data for B[a]P yields in reference cigarettes are well established [3-4]. The Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) recommended method for B[a]P [4] is the standardised method ISO 22634 [5]. Standardised smoking conditions are defined in ISO 3308 [6]. Other smoking protocols such as the Health Canada Intense (HCI) [7] aim to more closely reflect potential maximum levels. Published data for the other PAHs on the FDA list is somewhat limited and a comprehensive survey has not been published thus far.

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 (Borgwaldt KC, Hamburg, Germany) compliant with ISO 3308 [6]. Materials used for smoking and extraction are outlined in ISO 22634 [5]. The analysis was performed using GC-MS/MS (7890GC-7000MS/MS; Agilent, Santa Clara, CA, USA).

Sample Preparation

All cigarettes were conditioned in accordance with ISO 3402 [8]. Smoking was carried out in accordance with ISO 3308 [6] and HCI [7] protocols. Smoking parameters are outlined in Table 2. For HCI smoking all filter ventilation holes were blocked. Total particulate matter (TPM) values were obtained for all of the samples in accordance with ISO 4387 [9]. The extraction and clean-up procedure is outlined in ISO 22634 [5].

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

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.

Five unspecified branded cigarettes (Samples A-E) 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.

Results and Discussion

K3R4F Yield Data

The PAH yields along with the standard errors are shown in Table 3. There were 15 quantifiable compounds. Only dibenzo[a,l]pyrene (dB[al]P) was determined to be below LOD. B[a]P levels were consistent with published data. Naphthalene was by far the most abundant but also quite variable under ISO conditions. Generally mean yields were more variable under ISO conditions. Yields under HCI conditions were generally 2 to 3 times higher compared with ISO. The higher yields, hence more signal, may explain the greater precision observed.

Table 3. PAH mean yields in the K3R4F under ISO and HCI 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	365.2	74.7	1594.8	153.0
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
Dibenz[a,h]anthracene	0.66	0.10	1.73	0.15
Dibenzo[a,l]pyrene	< LOD	N/A	<LOD	N/A
Dibenzo[a,e]pyrene	0.24	0.03	0.62	0.12
Dibenzo[a,i]pyrene	0.17	0.04	0.39	0.10
Dibenzo[a,h]pyrene	0.16	0.04	0.33	0.07

Limit of Detection/Quantification (LOD/LOQ)

Repeat analyses of the lowest calibration standard were used to calculate LOD/LOQ (Table 4). It is an empirical measurement based on a procedure outlined in the Health Canada official method for the determination of B[a]P in cigarette MSS [10]. 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 4. Limits of Detection/Quantification (LOD/LOQ)

Analyte	ISO LOD (ng/cig)	ISO LOQ (ng/cig)	HCI LOD (ng/cig)	HCI 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
Dibenz[a,h]anthracene	0.02	0.08	0.05	0.16
Dibenzo[a,l]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

Branded Cigarettes and Other Monitor Data

A comparison of PAH yields between 5 branded cigarettes and 3 reference cigarettes is shown in Table 5. In all products only dB[al]P was determined to be below LOD. 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 [11]. 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.

Table 5. 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,l]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

Conclusion

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. Triple quadrupole MS provides a reliable means to analyse these components without employing complex clean-up procedures.

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