

CONFIDENTIAL

# UK SMOKE CONSTITUENTS STUDY

## ANNEX B

### Part 4 Validation data: Determination of Benzo[a]pyrene yields in mainstream cigarette smoke by gas chromatography - mass spectrometry

COMMISSIONED BY :

Tobacco Manufacturers Association

55 Tufton Street

LONDON SW1P 3QL

Report Number GC15/M26/02

Report Date July 2002

Contact Point: IAN AXFORD

Tel: 020 8943 7375

Prepared by: Pete Houlgate

Consumer Safety & Tobacco Products Group

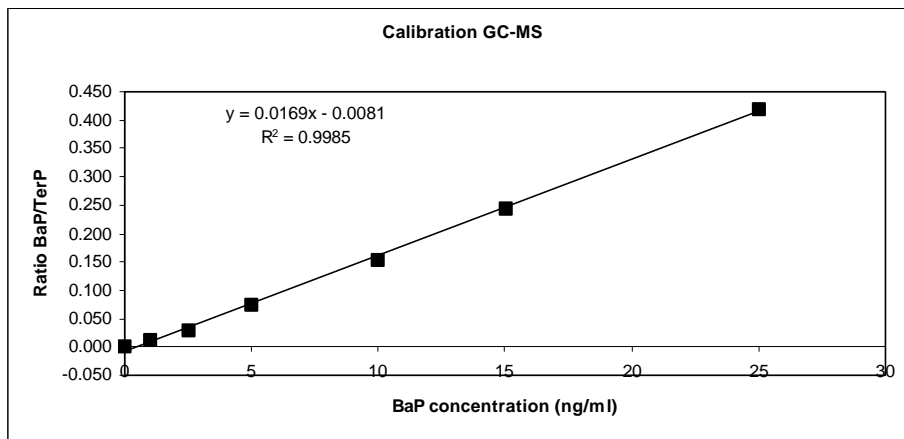


*Setting standards  
in analytical science*

## Validation data for benzo [a] pyrene

### 1. Calibration

After some initial trials, the GC-MS was calibrated for the range 1 to 25 ng mL<sup>-1</sup>, this being most appropriate for the range of benzo[a]pyrene yields to be encountered in the study.



### 2. Limit of detection/quantitation/reporting

Analysis of blanks (1R4F unlit cigarette) gave no detectable peak. When the data was processed a positive result was obtained due to the negative intercept on the calibration curve. The limit of detection was found to vary with each calibration. Therefore we did not determine a limit of detection but used the lowest concentration standard as the concentration to set a limit of quantitation (1 ng mL<sup>-1</sup>  $\equiv$  0.33 ng cig<sup>-1</sup>). The lowest standard gave a large enough peak to be detected and measured but repeatability could be poor depending on the sensitivity of the system. NB The sensitivity of the system deteriorates with time when analysing cigarette sample solutions – periodically the detector has to be cleaned to restore the sensitivity of the system. Therefore a reporting limit was used of <1 ng cig<sup>-1</sup> was used in the study

### 3. Precision and accuracy

Published results from the literature for benzo[a]pyrene yields in cigarette smoke for 1R4F are listed in the following table.

	Gmeiner <sup>i</sup>	Risner <sup>ii</sup>	Dumont	Risner <sup>iii</sup>	Tomkins <sup>iv</sup>	Evans <sup>v</sup>
	<----- ng per cigarette ----->					
Benzo[a]pyrene	7.9	9.2	8.5	6.4	6.6	6.9

Precision within a run for 1R4F and 1R5F was determined:

1R4F ( $\mu\text{g cig}^{-1}$ ): 6.30, 5.76, 7.11, 6.03, 6.17 (mean = 6.27, sd = 0.51, CV = 8.1%)\*

1R5F ( $\mu\text{g cig}^{-1}$ ): 1.61, 1.90, 1.92, 1.93, 1.71 (mean = 1.81, sd = 0.14, CV = 7.9%)\*

The precision achieved for 1R4F and 1R5F in the study (i.e. between runs) was:

1R4F ( $\mu\text{g cig}^{-1}$ ): 8.65, 6.67, 6.97, 6.70, 6.38 (mean = 7.07, sd = 0.91, CV = 12.8%)

1R5F ( $\mu\text{g cig}^{-1}$ ): 2.60, 1.66, 1.53, 1.42 (mean = 1.80, sd = 0.54, CV = 29.9% [n=4] )

\*Recoveries ranged from 55 % to 75% for the initial work on repeatability - theoretically 100% should be achieved. The reason for poor recoveries was identified and the method amended before carrying out the study.

Accuracy was found to be very dependent on the slope (and intercept) obtained from the calibration curve – see Section 4.

#### 4. Quality control

The results were found to be dependent on the slope and intercept of the calibration curve, which could change when analysing significant numbers of smoke solutions or between runs. The QC solution (nominal concentration 20 ng mL<sup>-1</sup> benzo[a]pyrene) was run after every 5 samples to check the calibration. Similarly a solvent blank was used to monitor the intercept of the calibration curve. If either went outside the limits the whole smoking run/analysis was repeated.

Additionally a smaller aliquot of sample solution was taken with high NFDPM yield cigarettes to minimise sample matrix effects on the performance of the GC-MS.

#### 5. Internal standard variation

It is noticeable that the area of the internal standard peak detected by the GC/MS varies depending on the sample matrix. For example, the internal standard peak areas measured for the blank, QC and standard solutions within a run were significantly smaller than for the smoke solutions.

A 1R4F sample solution in a GC vial, which had been through the cartridge clean up, was evaporated down to dryness. Solid material was observed in the vial at the end of the experiment.

An alternative clean up technique was used to try and identify what is happening. The clean up used was an HPLC instrument, normally used for cleaning up crude oil samples to identify and quantify hydrocarbon profiles. Results obtained for 1R4F were similar to those obtained for the cartridge clean up (6.0 & 6.5 ng per cig<sup>-1</sup>) and the internal standard area was similar to that achieved for the standards. However, recoveries were much lower (<10%) which meant

that the concentration of the solution injected onto the GC was ca 2 ng mL<sup>-1</sup> very close to the bottom standard on the calibration curve.

In our opinion the variation in internal standard area is dependent on the amount of material present in the sample solution.

## 6. Extraction efficiency

It is noticeable that the pads still have a lot of material on them at the end of the extraction process. Various experiments were tried to show that benzo[a]pyrene was being fully extracted from the pad – (a) pads were sonicated for 15 minutes, (b) pads were re-extracted with second batch of cyclohexane, (c) pad was put in rotary evaporator, (d) pads were spiked with BAP before and after smoking.

- (a) No significant difference was observed with sonication.
- (b) Some benzo[a]pyrene was found in the second extract solution (ca 10%) – however this was to be expected as not all the initial extract solution can be removed before the addition of the “second wash”.
- (c) No significant difference was observed in the sample concentration when pad extracted using the rotary evaporator.
- (d) Spiking experiments were conducted by adding 25 ng to the pad (5 ng per cig) before smoking). Results are shown in the table below:

Sample	Benzo[a]pyrene concentration ng per cig	Estimated recovery using mean values
Spike + 1R4F	11.43 & 12.15	110%
Spike + 1R5F	6.98 & 6.77	101%
Spike + blank pad	6.15 & 6.25	118%

<sup>i</sup> Gmeiner G et al, Determination of 17 polycyclic aromatic hydrocarbons in tobacco smoke condensate, *J. Chromatography A*, 1997, **767**, 163 - 169

<sup>ii</sup> Risner CH, Determination of benzo[a]pyrene in the total particulate matter of cigarette smoke, *J. Chromatogr. Sci.*, 1988, **26**, 113

<sup>iii</sup> Risner CH, Determination of benzo[a]pyrene and benzo[a]anthracene in mainstream and sidestream of smoke of Kentucky Reference cigarette 1R4F & a cigarette which heats but does not burn tobacco, A Comparison, *Beit. Z. Tabakforsch Int.*, 1991, **15**, 11-17

<sup>iv</sup> Tomkins BA et al, Liquid-chromatographic determination of benzo[a]pyrene in total particulate matter of cigarette smoke, *J Assoc. Off. Anal. Chem.*, 1985, **68**, 935-940

<sup>v</sup> Dumont J et al, Alternative isolation procedure for the subsequent determination of benzo[a]pyrene in total particulate matter of cigarette smoke, *J-Chromatogr-Sci.* 1993; **31**, 371-374