

Determination of Benzo[a] pyrene and Benzo[k] fluoranthene in Airborne Particulates

1. INTRODUCTION

Benzo[a] pyrene and benzo[k] fluoranthene are representatives of a class of polycyclic aromatic hydrocarbons some of which, including benzo[a] pyrene (BaP), are carcinogenic. Such hydrocarbons may be taken as indicators of the hazard which exposure may present. In addition to the fluorescence method to be outlined, other methods have been described, based on gas liquid chromatography, thin layer chromatography and ultraviolet absorption (12.1, 12.2, 12.3). A Manual of Methods is being prepared by the International Agency for Research on Cancer, in conjunction with the International Union of Pure and Applied Chemistry (12.4).

2. SCOPE

Airborne particulate matter is collected on suitable filters, the organic material is extracted with a suitable solvent and fluorescence measurements are made on chromatographed fractions.

3. FIELD OF APPLICATION

The method is applicable to airborne particulates collected from ambient air, industrial atmospheres or motor vehicle exhaust.

4. PRINCIPLE

A portion of the organic extract of an air sample is chromatographed with an aromatic solvent, toluene, on a column of alumina which has been activated at 140°C. The concentration of BaP in the eluates is determined by fluorescence emission measurements. The aromatic solvent is used in preference to an aliphatic or a saturated cyclic, since this results in a more rapid chromatographic separation.

5. REACTIONS

It is to be noted, in optical fluorescence, that the wavelength positions for maximum excitation and emission spectra vary with the solvent.

6. REAGENTS

6.1 *Cyclohexane, spectrograde*

Material suitable for either fluorescence or ultraviolet assay is readily and cheaply prepared by percolating a good technical grade through a column of activated carbon. The purified material is stored in glass stoppered borosilicate bottles. Technical cyclohexane produced from benzene is unsuitable as starting material as the high benzene content makes it too expensive to purify.

6.2 *Activated carbon*

A suitable grade is available from Pittsburgh Chemical Co., Pittsburgh, Pennsylvania. A column of the 12 x 30 grade, 6.0cm in diameter and at least 45cm in depth, allows the preparation of spectrograde cyclohexane at the rate of several litres per hour.

6.3 *Alumina*

Activated alumina is prepared by heating 100-200 mesh material to 140°C for 24 hours. A satisfactory grade is Peter Spence type H.

6.4 *Toluene*

Reagent grade, with a low fluorescence blank.

6.5 *Benzo[a]pyrene*

Is available from Fluka AG, Buchs, Switzerland or the WHO Reference Centre, Ottawa, Canada. CAUTION: THIS MATERIAL IS CARCINOGENIC!

6.6 *Benzo[k]fluoranthene*

This material is not available commercially. Limited quantities may be obtained for instrumental calibration from the WHO Reference Centre, Ottawa, Canada.

6.7 *Standard dilutions of BaP*

Prepare solutions of BaP in fluorescence-free toluene to contain 0.005, 0.010, 0.015, 0.020 and 0.025 microgram per ml of final solution.

6.8 *Standard dilutions of BkF*

Prepare solutions of BkF in fluorescence-free toluene, to contain 0.005, 0.010, 0.015, 0.020 and 0.025 microgram per ml of final solution.

7. APPARATUS

7.1 *Soxhlet extractors*

Extractors with a nominal solvent volume of 30ml and a cycling time of 2 minutes are used (12.5).

7.2 *Chromatographic columns*

Glass chromatographic columns with integral solvent reservoir at top and teflon plug stopcock are convenient. Internal diameter should be 10.0mm over a length of 30cm.

7.3 *Fluorimeter*

A fluorimeter with motor driven excitation and emission monochromators is required. This should cover the wavelength range of 250 to 550nm.

8. SAMPLING AND SAMPLES

Air samples are taken by drawing air at known flow rate and time through filters of cellulose or glass fibre. It is important that the filters used be free of organic extractable material which might fluoresce at the excitation wavelengths used. It has been found that small quantities of such fluorescing material are actually present in the commercially available material and it may be necessary to extract these materials from the filters before use. High volume samples may be taken for 24 hours in glass or cellulose filters 8 x 10 inches in overall size. If the hydrocarbon-in-air concentrations are high enough, smaller volumes of air may be taken on 4inch or 2inch filter circles.

9. PROCEDURE

Using a clean circular metal punch, cut 4 to 10 circles of 36mm diameter from a high volume filter, or a suitably large aliquot from low volume

filters. In the calculations given later, the factor 10.5 refers to an area aliquot equal to one-tenth of the total sample. Place the aliquot discs in a Soxhlet extractor on top of a wad of glass wool and cover with another wad of glass wool to lessen the possibility of the carryover of carbon particles into the extract. After 6 hours extraction with cyclohexane, evaporate the solvent extract carefully to 2ml, at room temperature, using a clean air or nitrogen jet but no heat. The extract must on no account be allowed to become dry.

Set up the chromatographic columns in a fume hood. Carry out the preparation of column and the elution procedure in the fume hood. Prepare the column by slurring the alumina with toluene and filling the tube to a depth of 12.0cm. Place the concentrated extract, no more than 2ml, carefully on top of the alumina which has been covered with a 1cm depth of glass beads. Elute, using toluene. Discard the first 25ml of eluate and collect 3ml fractions, thereafter, up to a total of about 30 fractions, or about 90ml. Scan each fraction separately on the fluorimeter and combine those fractions containing BaP and BkF for a further measurement. BaP and BkF are usually eluted between 30-45ml but this can vary depending upon the activity of the particular batch of alumina in use. Combine all fractions showing BaP and BkF, evaporate carefully without heat, and make up to a final volume such that the concentrations of BaP and BkF fit the range of the standard curves. See Figure 1. Usually a 5ml volume is satisfactory. Determine the fluorescence emission at 410nm by using the height-above-baseline technique, while exciting at wavelengths 309 and 385nm. These "optimum" wavelengths were selected by running the excitation spectrum of the 0.015 μ g/ml standards for both BaP and BkF. Four curves are obtained for solutions in toluene as shown in Figure 2 (Pages 276, 277).

10. EXPRESSION OF RESULTS

10.1 Calculations

Since the fluorescence emission intensity of BkF is much greater than that of BaP when a solution containing both hydrocarbons in equal amount is excited at 309nm, the reading at this wavelength is essentially due to BkF. Having determined the concentration of BkF, one can calculate the effect of this hydrocarbon when a mixture is excited at 385nm, after which the BaP concentration may be calculated in micrograms per ml, as follows:

$$\text{conc. BkF} = \frac{\text{Emission at 309 exc.}}{\text{Slope of BkF curve at 309 exc.}}$$

$$\text{conc. BaP} = \frac{\text{Emission at 395} - \text{conc. BkF} \cdot \text{slope BkF curve 385}}{\text{Slope of BaP curve at 385 exc.}}$$

10.2 *Pro forma calculation*

Suppose an eluate of an unknown sample to give a peak height of 14.8 units at 385 exc. and 38.5 units at 309 exc. By reference to the calibration curves in Figure 2, the slopes, or the emissions per μg of compound are:

$$\text{BkF (309)} = 8,800$$

$$\text{BkF (385)} = 1,850$$

$$\text{BaP (385)} = 3,850$$

From which the concentration of BkF = $\frac{38.5}{8,800} = 0.0044\mu\text{g/ml}$

And the concentration of BaP = $14.8 - \frac{(0.0044 \times 1,850)}{3,850} = 0.0039\mu\text{g/ml}$

Concentration of Hydrocarbon, $\mu\text{g/gram} =$

$$\frac{\mu\text{g/ml} \times 5 \times 10.5}{\text{particulate weight, grams}}$$

Concentration of hydrocarbon, $\mu\text{g/1,000m}^3 =$

$$\frac{\mu\text{g/ml} \times 5 \times 10.5 \times 1,000}{\text{air volume, m}^3}$$

10.3 *Accuracy*

0.250 $\mu\text{g/ml}$ of BaP can be measured with an accuracy of better than ± 0.002 microgram. 0.250 $\mu\text{g/ml}$ of BkF can be measured with an accuracy of better than $\pm 0.001\mu\text{g}$. If the concentration of BaP found is more than twice the BkF concentration, the BkF results will be in error by a factor of 10%.

11. NOTES ON PROCEDURE

11.1 *Collection efficiencies*

Little is known about actual collection efficiencies and in reporting the analytical data these efficiencies are usually ignored. Preliminary studies suggest that BaP is associated with very small particles and that collection efficiency increases with colder ambient temperatures.

11.2 *Temperature and pressure*

The effect of these sampling variables has been largely disregarded in the past. Since these factors must have some effect on the accuracy of the measured air volume, it is recommended that these corrections be

introduced routinely. There is increasing agreement, that if universal reference conditions of temperature and pressure are to be used to correct the final volume, these conditions should be 25°C and 760mm Hg.

11.3 Although the method described is nominally based upon "high volume" samples taken for a 24-hour sampling period, there is no valid reason why samples may not be taken for shorter time periods, at slower flow rates, or both. Lower particulate loadings obtained with smaller air volumes minimize particulate fall-off.

11.4 It is customary to assume that the decrease in air flow rate during sampling is linear and for usual purposes of calculation, the mean value of initial and final flow rates is taken. This is not correct, although the error introduced by this oversimplification is not ordinarily serious.

11.5 For the preparation of areal test portions, the use of a sharp circular metal die is to be preferred over the use of scissors or rectilinear templates of large area because of greater reproducibility and accuracy of the areal aliquot obtained.

11.6 In the use of replicate and consecutive areal test portions cut from an exposed high volume filter, the assumption is implicit that the material of interest is uniformly distributed across the exposed surface of the filter. This can be accepted as fact in the case of "high volume" filters (12.6). This uniform distribution cannot be assumed in certain "membrane" type filters due to inherently high pressure drop and faulty geometry of commercially available filter holders. In such case, it is necessary to use the complete filter (12.7).

11.7 *Sample preparation*

Soxhlet extraction, using organic solvents, is most widely used to obtain the extract of the organic material in the airborne particulates. Sublimation procedures, which require no solvent, are worthy of consideration (12.8). Recently, ultrasonic agitation of the sample in the presence of a solvent is said to be very rapid and effective. The procedure should be designed to avoid evaporation as much as possible, because of volatility losses. Heat should not be used and extracts should never be allowed to become dry. There is no blank problem with good quality glass or cellulose filters but, as mentioned, the absolute collection efficiency is unknown.

11.8 *Optimum wavelengths*

The optimum wavelengths may vary somewhat depending upon the instrument used. The values given are intended only as guides, since, because of instrumental variation, each laboratory must establish the optima which are obtained under their standard conditions. Calibration curves similar to

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Figure 2 are obtained for standard solutions in cyclohexane. The sensitivity is somewhat less in toluene than in cyclohexane. However, the toluene-based BaP measurement is somewhat better than a BaP measurement made in cyclohexane because the emission of BaP at 385 exc. is higher than the emission of BkF at 385 exc.

12. REFERENCES

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13. ANNEXES

Figure 1. Fluorescence emission spectra for standard polycyclic hydrocarbons compared with the spectra obtained on air sample extracts. This illustrates the so-called baseline method of peak height measurement (Page 276).

Figure 2. Four standard curves are given for benzo[a]pyrene and benzo[k]fluoranthene solutions in toluene as each standard solution is extracted using the extraction wavelengths 309 and 385nm (Page 277).





