

Liquid Chromatographic Method for the Determination of Benzo[a]pyrene in Filter Tar of Turkish Cigarettes

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An extraction method for benzo[a]pyrene determination in filter tar has been tried. Various Turkish cigarette filter tars were extracted using different solvents. The benzo[a]pyrene identification and quantification were carried out by means of high-pressure liquid chromatography. By the proposed method a recovery of 50% has been reached. Analysis of various Turkish cigarette filter tars showed that an average 78 ng/cigarette of benzo[a]pyrene is present.

Keywords: High-pressure liquid chromatography; cigarette; benzo[a]pyrene

INTRODUCTION

It is known that depending on the type of cigarette and smoking parameters, various classes of organic compounds are formed during smoking (Risner and Cash, 1990; Rogge et al., 1994). Some of these leave the cigarette together with the smoke, while some remain in the filter as a form of tar. Among the various organic compounds, such as alcohols, carbohydrates, aldehydes, ketones, and acids, polycyclic aromatic hydrocarbons (PAH) have a special place because of their carcinogenic and mutagenic activities. Therefore, their presence in the environment is not desired.

Benzo[a]pyrene (BaP) is a PAH for which the carcinogenic effect is known (Harvey, 1985). Its determination in various samples together with other PAHs has been carried out using different techniques. Spectroscopic and chromatographic techniques are generally used (Simonich and Hites, 1994; Copper and Sepaniak, 1994; Pinto et al., 1994; Potter and Pawliszyn, 1994). However, during recent decades high-pressure liquid chromatography has been preferred to spectroscopic and other chromatographic techniques. HPLC, especially in reversed phase mode, provides a unique selectivity for the separation of PAHs (de Kruijf et al., 1987), although the retention mechanisms in both reversed and normal phases are still under discussion (Fetzer, 1993). Another advantage of HPLC is that it can be used in preparative analysis, which is quite necessary for the analysis of complex mixtures such as filter extracts. Another advantage of HPLC is that it can be coupled to specific detectors such as a fluorometer, which is quite selective against PAHs. Recently developed supercritical fluid chromatography has been applied to PAHs as a detection and as an extraction method (Reindl and Höfler, 1994; Ono et al., 1981). PAHs have been determined quantitatively in various samples using either HPLC/UV spectrometry (Colmsjö and MacDonald, 1980), HPLC/spectrofluorometry (Pinto et al., 1994) or HPLC/GC (Kelly et al., 1993). To determine organic compounds formed during smoking, smoke condensate generally is analyzed (Snook et al., 1975). Even to see the effectiveness of the cigarette filter, most analyses were performed on smoke (Ono et al., 1981).

Very little research has been conducted on filter tar. The cigarette filter is an interesting sample to be monitored on the basis of the following arguments. (1) How effective is the filter? (2) How risky is it to hold the butt between the lips? (3) What is the rate of formation of BaP and other PAHs during smoking? These are some of the questions still to be answered.

In this work we have tried to develop a simple method for the extraction and determination of benzo[a]pyrene in cigarette filter tar, and we applied the method to some Turkish cigarettes.

MATERIALS AND METHODS

Cigarettes were bought from the free market randomly. BaP was purchased from Community Bureau of Reference Materials (Brussels). *n*-Hexane (Merck) was of chromatographic grade, while acetonitrile (Merck) and dichloromethane (Merck) were of analytical grade. Reversed phase column was a LiChrosorb C₁₈ (Chrompack); it has a 5 μm particle diameter with a surface area of 200 m²/g. Cigarette filters were prepared by means of a home-made automatic cigarette smoking machine. Smoking conditions were as follows: 2-s puff, 1 puff/min, 23-butt length, puff volume of 35 mL. The high-pressure liquid chromatograph was from Varian, Model 5500, equipped with a single-piston high-pressure pump having a pumping capacity of 0.1–10 mL/min. The chromatographic column was stainless steel with 25 cm length and 4.6 mm internal diameter. The detector is an internally built up UV spectrometer (Varian 200 UV) combined with a Packard, Model 642, recorder having a sensitivity of 10 mV/full scale deflection. For the recovery studies a smoked cigarette filter was spiked with 100 μL of standard BaP (6.75 μg/mL, in dichloromethane). Several extraction procedures were tried. Each was threefold. Before extraction, the remaining unsmoked part and then the filter paper were removed. The extraction procedures are outlined as follows:

a. Direct solid–liquid extraction using dichloromethane or *n*-hexane was performed as three successive extractions with 10 mL of solvent followed by concentration by means of a rotary vacuum evaporator. Each was injected to reversed phase HPLC.

b. Soxhlet extraction was performed with *n*-hexane and dichloromethane successively (each for 12 h). Concentrated extracts were injected to HPLC.

c. Successive extractions with *n*-hexane, dichloromethane, and methanol were each performed three times with 10 mL of solvent, followed by 10 min of vigorous shaking (without acetonitrile dissolution of filter).

d. Successive extractions with *n*-hexane, dichloromethane, and methanol were performed after dissolution of filter in

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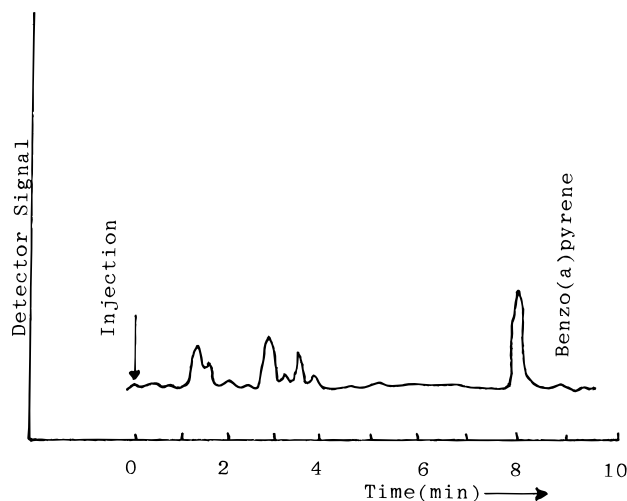


Figure 1. Chromatogram of the extract of a single cigarette filter tar spiked with standard BaP. (Chromatographic conditions are given in the text.)

Table 1. Extraction Recovery According to Method e (Four Stepwise Extractions with *n*-Hexane; Four Extractions)

| extraction step | % recovery | extraction step | % recovery |
|-----------------|------------|-----------------|------------|
| I | 29.04 | IV | 1.47 |
| II | 14.52 | | |
| III | 5.89 | total | 50.92 |

acetonitrile. Each extraction was carried out with 10 mL of solvent and followed by 10 min of vigorous shaking.

e. *n*-Hexane extraction was performed after the filter was dissolved in 10 mL of acetonitrile, at pH 2. Each time, 10 mL of solvent was added and the sample was shaken for 10 min thoroughly. In this method, first the filter paper was removed as mentioned above. The cellulose filter was dissolved in 10 mL of acetonitrile. The pH was adjusted to 2 by adding 0.5 M HCl. The extraction was carried out with 10 mL of *n*-hexane while shaking vigorously for 15 min. After the hexane phase was removed, another 10 mL of solvent was added. Following the third extraction three aliquots were collected and evaporated to dryness by means of a rotary vacuum evaporator. To the residue was added 100 μ L of dichloromethane, and of that, 10 μ L was injected to HPLC under the following chromatographic conditions: mobile phase, 84% acetonitrile/water; flow rate, 2 mL/min, at 160 atm; detector, 254 nm, 0.01–0.001 abs; recorder, 10 mV/full scale deflection; chart speed, 0.5 cm/min.

Identification and quantification of the BaP peak were done by injecting standard BaP solution and comparing the peak areas. Table 1 shows extraction recoveries of method e with a percent relative standard deviation of 10.5 (four extractions.)

By proposed methods a–d the extraction yield never exceeded 40%. Therefore, we have applied method e as the method of choice for quantitative analysis of real samples. Five cigarette filters were extracted according to procedure e. The results are shown in Table 2. Figure 1 gives the chromatogram of a single cigarette filter spiked with standard BaP (corresponding to 170 ng of BaP at an attenuation of 0.01 abs); Figure 2 shows a chromatogram of a five-cigarette extract at different attenuations, namely 0.1 and 0.001 abs.

RESULTS AND DISCUSSION

Recovery studies show that by the proposed extraction methods a–d extraction recovery never exceeded 40%. For instance, Soxhlet extraction with dichloromethane gave a recovery of 35%. Only the last method, namely, extraction with *n*-hexane after the filter was dissolved in acetonitrile, gave the best recovery percentages,

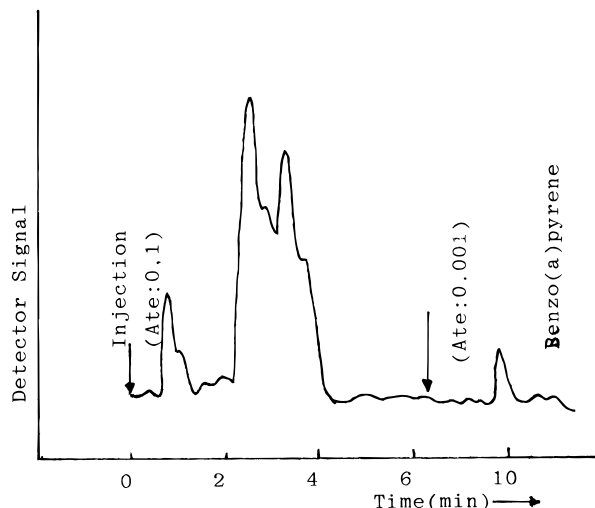


Figure 2. Chromatogram of the extract of five cigarette filter tars. (Chromatographic conditions are given in the text.)

Table 2. BaP Found in Various Turkish Cigarette Filters (Two Extractions)

| type | BaP, ng/filter | type | BaP, ng/filter |
|------|----------------|------|----------------|
| I | 85 | III | 98 |
| II | 51 | av | 78 |

reaching 50% with a percent standard deviation of 10.5. The reasons for low recovery can mainly be attributed to (1) the complex composition of the tar mixture, which is why even the best generally used solvent dichloromethane could not increase the percent recovery; and (2) adsorption of BaP on the glass wall (Pinto et al., 1994).

In Figure 1 we see that BaP is completely resolved from other compounds in the extract. Even when five cigarettes were extracted, baseline resolution could be reached, which makes the quantification easier. Under these conditions there is no need for a cleanup, although we have tried XAD-2, XAD-4, alumina, and silica adsorbents, which may still be useful for the resolution of other PAHs, which we are working on. The BaP content is very low. Remembering the lower detection limit of UV spectrometry (Giles et al., 1979), as low as 0.5 ng of BaP, by analyzing five cigarette filters, we were able to find 0.051 ng/filter BaP. The results of various Turkish cigarettes are presented in Table 2. The average of three sorts is 78 ng.

CONCLUSION

Although the recovery percent of BaP seems rather low, it is worth trying to improve the method in this direction, which is what we are doing. The method is being tried to determine other PAHs in filter and smoke.

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