[Chem. Pharm. Bull. 26(11)3600—3602(1978)]

UDC 547.689.08 : 543.544.02.2.062

Ultramicro Analysis of Benzo[*a*]pyrene in Food

Mass-fragmentography was used for the quantitative analysis of benzo[*a*]pyrene in food. Improvement of column packing materials for gas-liquid chromatography made an analysis of pico gram amount of benzo[*a*]pyrene possible. The procedure for the analysis is quite simple. Some experimental results were shown.

Keywords——benzo[*a*]pyrene; benzo[*e*]pyrene; GC–MS; mass-fragmentography; liquid crystal

Benzo[*a*]pyrene (BaP) is one of potent carcinogens and widely distributed in food and other environmental substances.[1] In general pico gram amount of BaP in food is analyzed by fluorescence spectroscopic[2] and other methods.[3] Though these methods are often used, the preparation of the test sample is troublesome, and in many cases the co-existance of other aromatic hydrocarbons makes the accuracy very poor. In this paper we wish to describe a

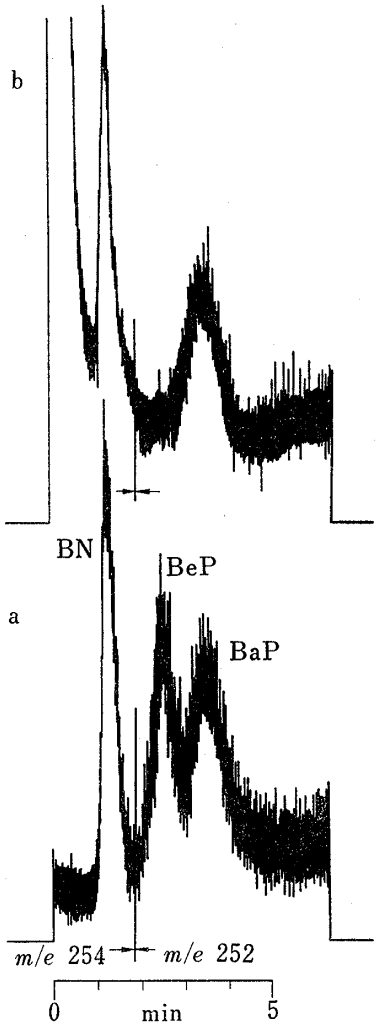

Fig. 1a. Chromatogram of a Mixture of BaP, BeP, and Binaphthyl (BN)

Sample: methylene chloride solution 1 μl, BaP (1 pg), BeP (1 pg), and binaphthyl (BN) (2 pg).
Column: alkali-BBBT-alkali, shown in the text.
GC conditions: Oven temp. 254°, injector and separator temp. 265°, flow rate 30 ml/min (He).
MS conditions: Ion sause temp. 290°, accelerating volt. 3.5 kV, electron energy 20 eV.
Target mass number was 254 and 252 before and after about two minutes, respectively.

Fig. 1b. Chromatogram of a Sample obtained from Soy Sause

Sample: methylene chloride solution 6 μl prepared in the procedure (2). The amount of BaP corresponds to about 0.8 pg.
Column and GC-MS conditions were same as Fig. 1a.

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3) K. Shiraishi, *J. Japan Oil Chemists' Soc.*, **25**, 721 (1976).

simple and reliable analytical method of BaP in food by the use of mass-fragmentography (specific ion monitoring by gas chromatography–mass spectrum (GC–MS).)[4]

A mixture of authentic materials containing BaP was quite well analyzed in the pico gram order by GC–MS using silicone oil[5] as the liquid phase of GC, though BaP could not be resolved from isomeric benzo[*e*]pyrene (BeP). However, a sample from various food after convenient work-up procedures could not be analyzed because of a large amount of back ground contaminants.

This problem can be much improved by connecting a short (5—10 cm) alkali-coated column at the injection part of the silicone-coated main column. The alkali column efficiently traps the back ground substances. This alkali column method is very effective in the analysis of polychlorobiphenyls from biomaterials using silicone oil.

Recently a successful utilization of a liquid crystal, N,N′-[*p*-butoxybenzyliden]-α,α′-bi-*p*-toluidine (BBBT) in its nematic range was illustrated for the resolution of geometric isomers of various polycyclic aromatic hydrocarbons.[6] This column was very effective in the separation of BaP from BeP. However the vapor pressure of the liquid phase is so high that the mass-fragmentographic analysis of a very small amount of BP can not be performed. Fortunately, the compound BBBT (or contaminants in BBBT) was completely trapped by a short packing of alkali-coated Chromosorb WHP at the out-put part of the separation column. The alkali column packed at the injection part also helped the analysis and saved the lifetime of the column.

Thus, the analysis of BP was preformed using a glass column (50 cm × 2.5 mm i.d.) packed succesively with 10% sodium hydroxide-Chromosorb WHP (5 cm), 1.5% BBBT-Chromosorb WHP (42 cm), and 10% sodium hydroxide-Chromosorb WHP (3 cm). The first alkali packing had better be replaced after every 30—100 injections of samples. The instrument used was a Shimadzu-LKB-9000 equipped with a multiple ion detector.

The column temperature was set around at 240°. Selected mass was $m^+/e=252$ (molecular ion of BP) and 254 (molecular ion of an internal standard, α,α′-binaphthyl). A chromatographic pattern of an authentic mixture of BaP, BeP, and binaphthyl was illustrated in Fig. 1a.

Table I. Analytical Results

Sample	BBBT method[a]		OV-101 method[b]
	Bap (ppb)	Bep (ppb)	BaP+BeP (ppb)
Green tea leaves	7.2	3.6	14.3
Burned wheat	7.6	*c*)	*c*)
Soy sauce	6.8	*d*)	13.8
Burned rice	4.2	1.6	6.1
Toasted bread	3.8	*d*)	*c*)
Solid soup	3.0	3.0	*c*)
Whisky	0.2	*d*)	*c*)

a) Condition: see the Fig. 1a.
b) Condition: column.
OV-101, 1.5% on Chromosorb W 90 cm+alkali 10% on Chromosorb W 10 cm. GC-MS. Oven temp. 270°, injector and separator temp. 300°, ion sause temp. 290°, flow rate 30 ml/min (He), accelerating volt. 3.5 kV, electron energy 20 eV. Tetraphenylmethan as the internal standard.
c) Not determined because of superimposed peaks or back ground.
d) Noise level amount.

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The detection limit was less than 1 pg (absolute amount of an injection). A good linear calibration curve against the internal standard was obtained through the injection amount of 0.5 pg to 5.0 ng of BP.

Two procedure of extracting BP from food can be used in the present method. The simplicity removes various errors which may accumulate during the sample preparations. (1) A solid sample (5—30 g) is digested in 40—100 ml methanol and 10 ml 10% sodium hydroxide. The mixture heated at 60° for 3 hr. After evaporation of methanol, BP is extracted with two 10 ml portions of methylene chloride containing the internal standard (10—30 ppb). The combined methylene chloride extract is concentrated to less than 1 ml, whose 5—10 μl is injected to GC–MS. In a very fatty sample, the amount of alkali is deficient, and some improvement in the procedure may be required (use of more solvent and more alkali). (2) A liquid sample, *per se* or after concentration, is extracted with 10 ml of methylene chloride containing the internal standard. Methylene chloride extract is concentrated to less than 1 ml, whose 5—10 μl is injected. Recovery of added BaP in soy sauce was more than 90%.

Some results were shown in Table. Mass -fragmentographic chart obtained in the analysis of soy sauce was illustrated in Fig. 1b. Amounts of BeP could be estimated in few cases. Rough amounts of the BaP and BeP by analysis using OV-101 as the liquid phase were also shown in Table for the sake of comparison. The evaluation of BP contents will be discussed after accumulation of the analytical data on various food. Application of the present method to other hydrocarbons may be promising.

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Received September 5, 1978