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Determination of Benzo(a)pyrene in Liquid Paraffin by High Performance Liquid Chromatography (HPLC)

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The potential presence of carcinogenic polycyclic hydrocarbons in various petrolium products for food additive use has stimulated interests in rapid sensitive and accurate method. In the case of petrolium waxes, methods have been developed for limiting the possible content of polynuclear carcinogens using absorption spectrometry. (E. Haenni et.al. 1962; W.Lijinsky et.al. 1963; J.W. Howard et. al. 1965) Because the majority of polynuclear hydrocarbons are not carcinogenic, methods which detect the specific carcinogens have been developed by the use of chromatographic techniques. Fluorescent spectrometry after the clean up with column and (or) thin layer chromatography has been commonly used for the determination of benzo(a)pyrene. The use of gas-chromatography equipped with electron capture detector is also reported. (W. Lijinsky et.al. 1965)

High performance liquid chromatography has recently been recognized to have advantages in sensitivity and specificity for the determination of individual homologues especially when it is connected to fluorescent monitor. (I.Berthold 1974; W.J. Chamberlain et.al. 1975) Presented here is an application of high performance liquid chromatography to routine analysis of 3,4-benzo(a)pyrene in liquid parafin which is widely used for food additive use.

Materials and Method

Examined liquid parafin samples were commercially available ones for food additive use. Solvents were the highest grade obtainable. Analysis was performed with HPLC (Waters' Associate) equipped with fluorescent monitor(Shimadzu RF 500LC).

Liquid parafin (10g) was dissolved in 10 ml of n-hexane and extracted three times with the portion of 10 ml dimethylsulphoxide(DMSO). DMSO solution was collected and 30 ml of water was added to DMSO solution and then extracted with n-hexane(10ml) three times. N-hexane extract was dehydrated with anhydrous sodium sulphate and concentrated to the volume of one mililitre with Kundera-Danish concentrator and nitrogen

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gas flashing. An aliquot of the hexane solution (20-50 µl) was injected to the HPLC. Chromatographic conditions were as folows.

Column mivro Bondapack C_{18} (lft 1/4 inch X 1/8inch)
Eluent Acetonitrile + Water (70:30) Flow 2ml/min.
Detector Shimadzu RF 500 LC Ex. w.l. 366 nm
Em. w.l. 406 nm

Result and Discussion

Selection of excitation and emission wave lengthes was important to get high sensitivity. By maximuming the peak height of Benzo(a)pyrene (B(a)p) on the chromatogram, excitation and emission wave lenghes were selected. The highest sensitivity was obtained in the combination of 366 nm excitation and 406 nm emission wave lengh. Linearity of recorder response was held in the range of 10^{-11} - $5x10^{-9}$ B(a)P. (Fig. 1)

Fig.2 shows a chromatogram of B(a)P and B(e)P. B(a)P was well separated from B(e)P. Resolution was about 1.3. By the use of polar solvent(AcCN 60: H_2O40) the resolution was improved but the time required for analysis was doubled. It was also noted that B(e)P was much less senssitive at the above wave lengthes than B(a)P indicating that the presence of B(e)P was practically negligible.

Replicated analyses of spiked samples in the level of 2 ppb($2x\ 10^{-9}$) B(a)P revealed that the mean recovery was 94 % with a small variation of 0.8%. Minimum detection limit in this procedure was 50 ppt ($50x10^{-12}$) in liquid parafin.

Fig. 3 shows a typical chromatogram of liquid parafin. B(a)P appears as one of the highest peaks on the chromatogram. The effluent corresponding to the B(a)P peak was collected and subjected to fluorescent analysis. (Fig.4) The emission spectra of the corresponding peak was quite similar to that of the authentic sample. Hence it seemed reasonable to neglect the interference by other substances.

Analytical data of eight commercial liquid parafins are given in Table 1.

Table 1. B(a)P level in commercial liquid parafin

No.	B(a)P	No.	B(a)P	No.	B(a)P	
1	0.20	4	0.21	7	0.30	
2	0.32	5	0.17	8	0.36	
3	0.20	6	0.26	Mean	0.25	(ng/g)

Although the level was low, B(a)P was detected in all samples we examined.

Fig.1 Calibration Curve

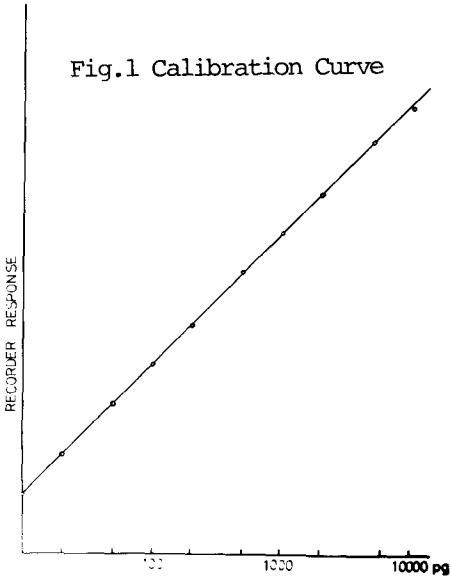


Fig.2 Separation of B(a)P and B(e)P

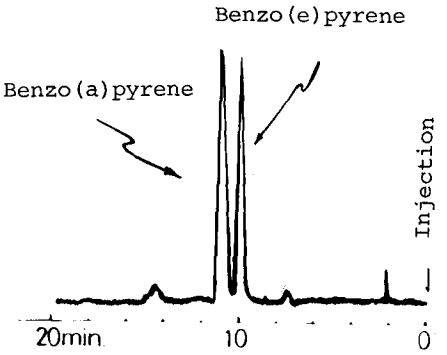


Fig.3 Chromatogram of Liquid paraffin

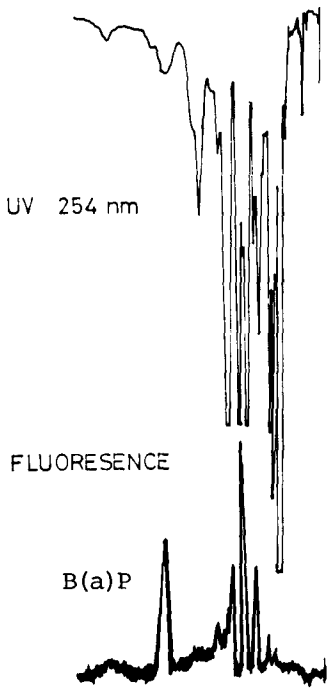
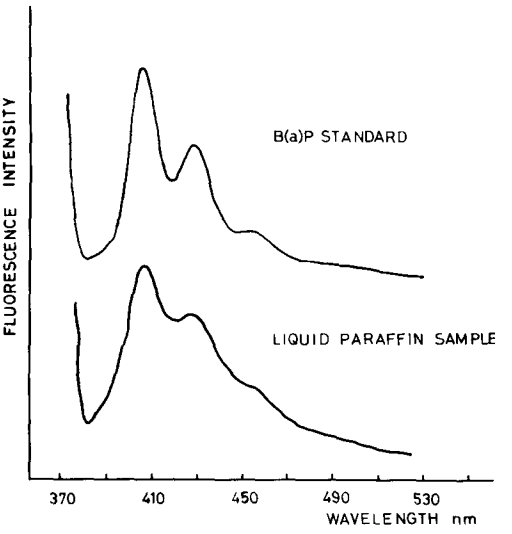


Fig.4 Emission spectra of B(a)P coresponding fraction and authentic sample.
Ex. w.l. 366nm



The present method seems more suitable for routine analysis of B(a)P in liquid parafin than those earlier reported. Since clean up procedures with chromatography are excluded in this method, one may be afraid that column life is shortened. Filtration with membrane filter (0.45μ) before injection and the conditioning by passing tetrahydrofuran through injection port and column after the measurement seemed effective to prevent the deterioration of the column: More than two hundred times of injection gave no apparent deterioration of the column.

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