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COMPARISON OF A CAPILLARY GAS CHROMATOGRAPHIC AND A HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD OF ANALYSIS FOR POLYCYCLIC AROMATIC HYDROCARBONS IN FOOD

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SUMMARY

Five food samples have been analysed for a number of polycyclic aromatic hydrocarbons (PAHs) by capillary gas chromatography with flame ionization detection (GC–FID) and high-performance liquid chromatography (HPLC) with fluorescence detection. The methods give similar results and there is no significant difference in their repeatability. The capillary GC method is favoured where it is desirable to analyse for a large number of PAHs, whereas the HPLC method is preferred for the individual analysis of a smaller number of PAH isomers.

INTRODUCTION

A very large number of methods have been developed for the analysis of polycyclic aromatic hydrocarbons (PAHs) in environmental samples and these have been extensively reviewed by Lee *et al.*[1]. These authors emphasise the importance of analysing individual PAH isomers because of their considerable variation in toxicity. For this reason the high peak capacity of capillary gas chromatography (GC) has made this technique an obvious choice in PAH analysis. Vassilaros *et al.*[2] have analysed fish samples using a 20-m SE-52 column with flame ionization detection (FID) and confirmed identities by mass spectrometry (MS). Grimmer *et al.*[3] have analysed water samples using a 25-m CpSil 5 column with FID. Bartle *et al.*[4], in reviewing this subject, find that SE-52 and SE-54 columns with FID have been the most popular capillary GC technique.

High-performance liquid chromatography (HPLC) has attracted a considerable amount of attention for use in PAH analysis. Although in peak capacity HPLC columns are considerably inferior to capillary GC columns, it is sometimes possible to perform critical isomer separations quite easily by HPLC where these separations are very difficult by capillary GC. In this regard Ogan and Katz[5] emphasised the

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value of polymeric reversed-phase HPLC columns such as Vydac over conventional C_{18} columns. The disadvantage of the relatively poor peak capacity of the HPLC system can be further offset by the use of sensitive and specific detectors. Choudhury and Bush[6] found on-line millisecond-scan LC–UV spectrometry a most valuable adjunct to GC–MS for achieving isomer-specific identification of PAHs and used these techniques for the analysis of air particulate samples. Many investigators have used the exceptional sensitivity and selectivity of fluorescence detectors for analysing PAHs in complex matrices. Additionally, the non-destructive nature of spectrophotometric detectors allows their use in series. Thus the identities of the analytes can be confirmed either by using similar detectors under different wavelength conditions or two detectors working on different principles. For instance, Crosby *et al.*[7] used two fluorescence detectors for the analysis of PAH in food, water and smoke, while Joe *et al.*[8] used fluorescence and UV detection for the analysis of PAH in barley malt.

May *et al.*[9] have used both GC–MS and HPLC–fluorescence to analyse a shale oil sample for use as a standard reference material. Both techniques provided very similar results for a range of PAHs in this sample, although some discrepancies were evident for a sample of urban particulate matter. In this paper a capillary GC–FID method and a HPLC–fluorescence method for the analysis of PAHs in food samples are compared.

EXPERIMENTAL

Sample preparation

The food samples were purchased and prepared in Uppsala. The sausage and pork chops were grilled (medium cooked) over a charcoal fire; a further sausage sample was grilled (well done) over an open wood fire. Samples of smoked ham and smoked herring were analysed without further cooking. Each sample was thoroughly mixed in a Moulinex mixer, freeze-dried and mixed again before samples were withdrawn for analysis.

The samples were analysed immediately in triplicate by the GC method and after 3 months in duplicate by the HPLC method. Except during transit between laboratories, the samples were stored frozen (−16°C) during this period. Just before analysis the samples were dried at 85°C for 2 h by both laboratories.

The sample work-up followed broadly similar lines for both analytical methods. The samples (10 g) were mixed with internal standard, digested in boiling methanolic potassium hydroxide, extracted into an aliphatic solvent and partitioned into dimethylformamide. After addition of water and extraction into the aliphatic solvent, a final clean-up on silica was performed. A detailed description of the work-up for the GC method is given by Larsson[10] and for the HPLC method by Dennis *et al.*[11].

Capillary GC analysis

The sample was analysed using a 50 m × 0.30 mm I.D. SE-54 glass capillary column with flame ionization detection. Samples (1–4 μl) were injected using an LKB falling-needle injector. Conditions used were: hydrogen carrier gas, 3 ml min^{-1}; nitrogen make-up gas, 30 ml min^{-1}; temperature programme, 165°C for 6 min then

4°C min^{-1} to 255°C. Individual PAHs were identified by comparing their retention times with those of known standards, and quantitated by comparing the integrated peak areas with that of the internal standard ($\beta\beta$-binaphthyl).

HPLC fluorescence analysis

The samples (20 μl) were analysed using a 5-μm Spherisorb ODS precolumn and a 5-μm Vydac ODS analytical column at 30°C. They were applied using a Rheodyne fixed-volume loop injector and eluted using a linear acetonitrile–water gradient, 60–90% acetonitrile over 35 min. Detection was by a Perkin-Elmer 3000 fluorimeter (excitation wavelength 290 nm, emission wavelength 430 nm) and by a Perkin-Elmer 1000M filter fluorimeter (excitation filter 340 nm interference, emission filter long pass cut-on at 390 nm) set in series. The amount of a particular PAH in a sample was determined by comparison of the peak heights with those from a known set of standards run on the same day under the same conditions. Recovery was determined using a perylene internal standard of sufficientlhigh concentration to mask any trace amounts of perylene in the sample

RESULTS AND DISCUSSION

The PAH contents of a number of food samples are recorded in Table I, and typical chromatograms of a sample and standards are shown in Figs. 1–4. Triplicate analyses were performed for the GC method and duplicate analyses for the HPLC method. The means and standard deviations (S.D.s) of these analyses are given and the results from the two methods are generally in good agreement. A statistical procedure (Student *t* test) was applied to test whether the difference between the sample means of the GC and HPLC methods was significant for comparable PAH analyses. (The data for benzo[*k*]fluoranthene/benzofluoranthenes and dibenz[*a,h*]-anthracene/dibenzanthracenes were not compared because different analytes were being measured by the two methods.) Thirty-five pairs of analyses were tested and 25 of these were not significantly different within the 95% confidence limits employed. The remaining pairs were distributed across a wide range of PAHs and in all food samples, suggesting that no systematic error is occurring. Indeed the correspondence between the GC and HPLC methods seemed empirically adequate for most of these analyses. For instance, the test indicated that benzo[*g,h,i*]perylene in smoked herring (GC 3.0 $\mu g\ kg^{-1}$, HPLC 1.2 $\mu g\ kg^{-1}$) and benzo[*b*]fluoranthene in grilled pork chops (GC 5.5 $\mu g\ kg^{-1}$, HPLC 4.0 $\mu g\ kg^{-1}$) were amongst those compounds showing the greatest probability that the two methods produced significantly different results. In fact these results would normally be considered sufficiently close for practical purposes.

Overall however, the standard deviations indicate that the repeatability of the two methods is very good, being usually within 10% of the mean, and there seems to be no major difference between the GC and HPLC methods. The methods appeared well able to provide comparable data throughout the wide range (0.2–1000 $\mu g\ kg^{-1}$) of PAH concentrations employed in this study. The comparability of the GC and HPLC data for benzo[*a*]pyrene in grilled and hard grilled sausage was noticeably poor. Since the agreement between the methods for benzo[*a*]pyrene was particularly good for the other samples it seemed possible that some change in sample

TABLE I

A COMPARISON OF CAPILLARY GC AND HPLC ANALYSES OF SOME FOOD MATERIALS FOR PAHs ($\mu g\ kg^{-1}$)

Compound	*Grilled sausage*		*Grilled pork chops*		*Smoked herring*		*Smoked ham*		*Hard grilled sausage*		*Detection limits*	
	GC (S.D.)	*HPLC (S.D.)*	*GC (S.D.)*	*HPLC (S.D.)*	*GC (S.D.)*	*HPLC (S.D.)*	*GC (S.D.)*	*HPLC (S.D.)*	*GC (S.D.)*	*HPLC (S.D.)*	*GC*	*HPLC*
Fluoranthene	15.9 (1.6)	12.8 (0.9)	57.4 (2.2)	58.3 (2.5)	106.5 (14.9)	90.1 (3.7)	9.4 (1.0)*	6.3 (0.3)	818 (14)*	1071 (93)	0.3	0.2
Pyrene	18.1 (1.8)	17.5 (1.3)	61.1 (1.3)	68.2 (6.5)	111.2 (12.4)	116.5 (4.2)	14.0 (2.3)	8.0 (0.1)	982 (24)	1410 (252)	0.3	2.5
Benz[*a*]-anthracene	1.9 (0.1)	1.6 (0.1)	8.2 (0.1)*	9.8 (0.6)	26.7 (1.9)	24.4 (0.1	0.4 (0.1)	0.4 (0)	314 (15)	309 (28)	0.2	0.08
Benzo[*e*]-pyrene	1.3 (0.1)	<1.7	5.4 (0.3)	4.8 (0.2)	4.8 (1.2)	<1.7	0.2 (0)	<1.7	176 (5)	254 (42)	0.2	1.70
Benzo[*b*]-fluoranthene	1.4 (0.3)	0.9 (0.1)	5.5 (0.1)*	4.0 (0.4)	6.4 (0.6)*	4.1 (0.1)	<0.1	0.1 (0)	201 (6)	192 (13)	0.1	0.05
Benzo[*k*]-fluoranthene		0.2 (0)		1.4 (0.1)		1.7 (0.2)		0.04 (0.01)		100 (2)		0.01
Benzo[*k*]-fluoranthene + benzo[*j*]-fluoranthene	0.6 (0)		6.7 (0.4)		10.1 (0.4)		<0.1		375 (14)		0.1	
Benzo[*a*]-pyrene	0.8 (0.1)*,**	0.4 (0)	8.6 (0.3)	8.2 (0.3)	8.4 (0.7)	8.5 (0.3)	0.2 (0)	0.2 (0.1)	460 (17)*,**	191 (7)	0.1	0.05
Benzo[*g,h,i*]-perylene	1.6 (0.2)*	0.9 (0)	9.5 (0.8)*	6.2 (0.1)	3.0 (0.2)*	1.2 (0.1)	<0.4	0.1 (0)	332 (19)	335 (25)	0.4	0.15
Dibenz[*a,h*]-anthracene		0.2 (0.1)		1.3 (0.1)		Not analysed		<0.1		22.5 (2.1)		0.10
Dibenz-anthracenes	<1.0		<1.0		<1.0		<1.0		16.2 (0.6)		1.0	

* Students *t* test indicates this analysis to be significantly different.

** Reanalysis showed that after 11 months this concentration had fallen to less than 20% of its initial level.

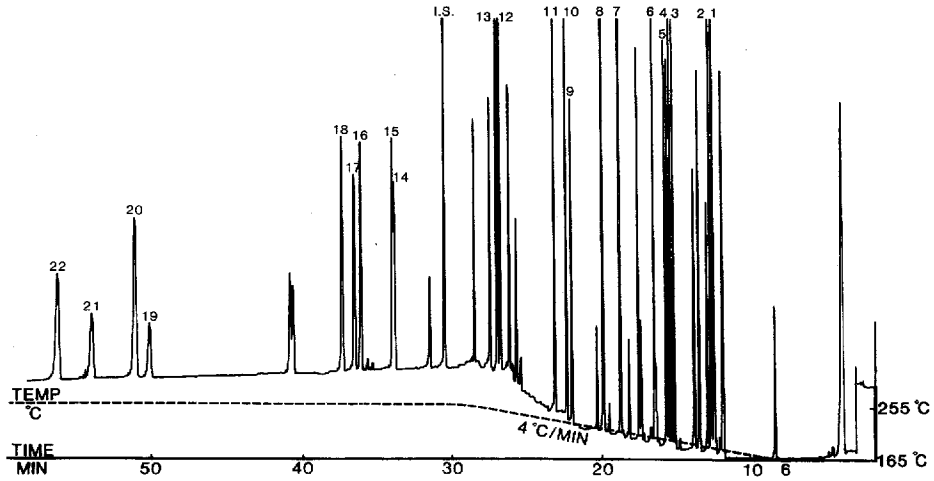

Fig. 1. Gas chromatogram of a PAH standard mixture containing phenanthrene (1), anthracene (2), 2-methylphenanthrene (3), 2-methylanthracene (4), 1-methylphenanthrene (5), 9-methylanthracene (6), fluoranthene (7), pyrene (8), benzo[*a*]fluorene (9), benzo[*b*]fluorene (10), 1-methylpyrene (11), benz[*a*]anthracene (12), chrysene and triphenylene (13), benzo[*b*]fluoranthene (14), benzo[*j*]fluoranthene and benzo[*k*]fluoranthene (15), benzo[*e*]pyrene (16), benzo[*a*]pyrene (17), perylene (18), indeno[1,2,3-*c,d*]pyrene (19), dibenzanthracenes (20), benzo[*g,h,i*]perylene (21), anthanthrene (22) and $\beta\beta$-binaphthyl (I.S. = internal standard).

composition had occurred in the 3 month interval between the two sets of analyses. Re-analysis of the grilled sausage samples by the GC method indicated a considerable loss of benzo[*a*]pyrene; after 11 months both samples contained less than 20% of the original level of benzo[*a*]pyrene and this loss of analyte seems the most likely reason for the discrepancy between the GC and HPLC results. Only minor changes in the levels of the other PAHs being compared had occurred during this period and these were within the repeatability of the analyses, so that no effect on the comparison of the analytical techniques is likely. Re-analysis of the grilled pork chop and smoked

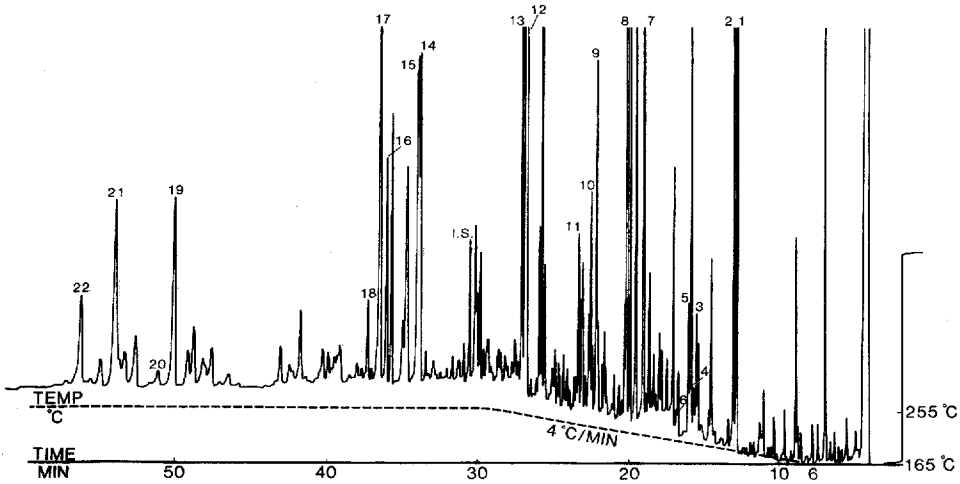

Fig. 2. Gas chromatogram of an extract of hard grilled sausage. PAH identification as in Fig. 1.

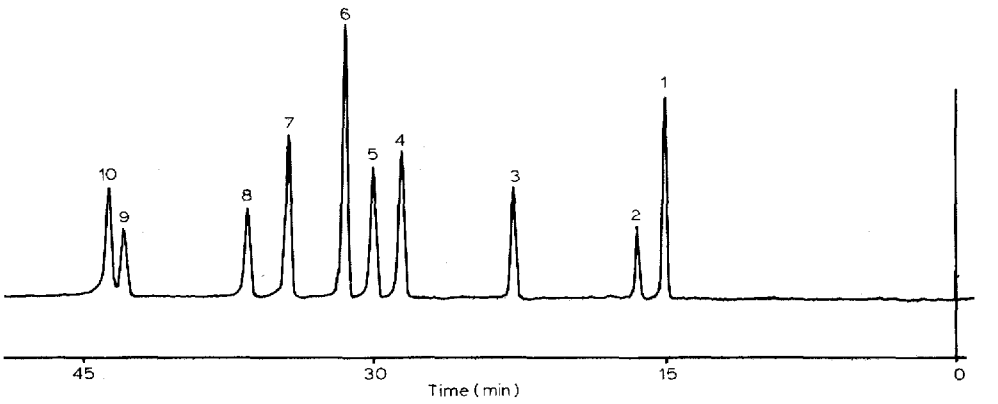

Fig. 3. HPLC analysis of a PAH standard mixture containing fluoranthene (1), pyrene (2), benz[*a*]anthracene (3), benzo[*e*]pyrene (4), benzo[*b*]fluoranthene (5), perylene (6), benzo[*a*]pyrene (7), benzo[*k*]fluoranthene (8), benzo[*g,h,i*]perylene (9) and dibenz[*a,h*]anthracene (10).

herring samples again indicated insignificant changes in PAH composition over this time period.

The analysis of benzo[*k*]fluoranthene by the two methods is not strictly comparable because benzo[*j*]fluoranthene is not separated from this isomer by the GC method. The higher figures obtained by the GC analysis are consistent with the presence of more than one analyte. This emphasises one of the major advantages of HPLC over capillary GC, namely the ability to separate many PAH isomers. The GC method is unable to resolve chrysene and triphenylene or the dibenzanthracenes (see Figs. 1, 2) whereas these separations are not normally a problem for HPLC[6]. The fluorescence detection system is also able to resolve these components and indeed

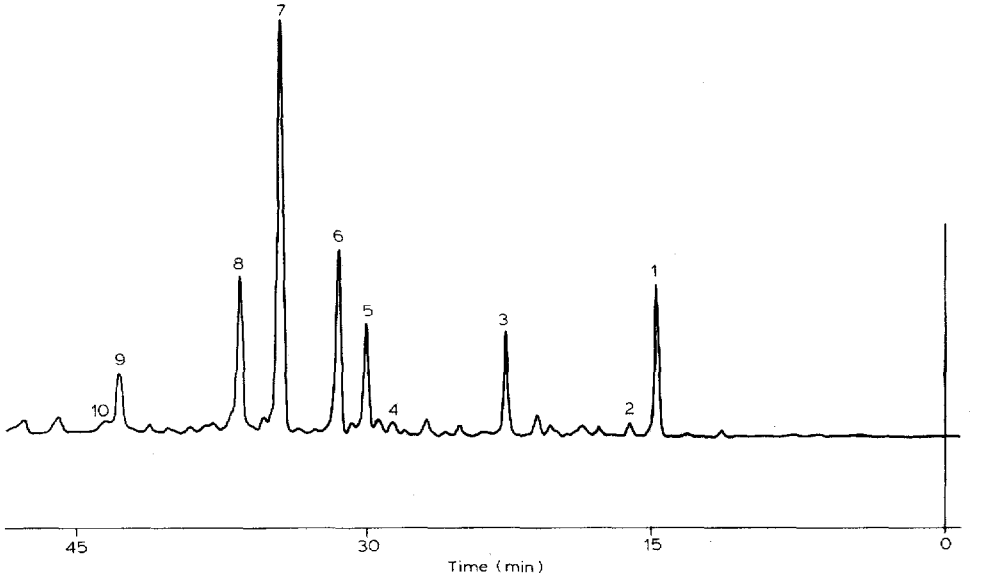

Fig. 4. HPLC analysis of an extract of hard grilled sausage to which perylene (internal standard) has been added. PAH identification as in Fig. 3.

triphenylene, benzo[*j*]fluoranthene and dibenz[*a*,*c*]anthracene are not detected under the fluorescence conditions used.

This fluorescence specificity has attendant drawbacks. While it helps to guarantee the correct identification of a peak (and this facility was extended in this study by the use of two fluorimeters set in series under different wavelength conditions), it also limits the number of compounds which can be analysed in a single analysis. Chrysene was not analysed by HPLC because of the relatively poor detection limit found under the fluorescence conditions used. Indeed the variability of the detection limits for the HPLC method is evident from Table I and is in contrast to the GC method where the detection limits are fairly similar for all PAHs. This difference reflects the difference in specificities of the FID and fluorescence detection instruments and the wide response shown by FID is most useful in the GC method.

The capillary GC column possesses a much greater resolving power, in terms of plate number, than the HPLC column so that many more compounds can be separated and detected by FID. Fig. 2 shows a chromatogram of the hard grilled sausage sample analysed by capillary GC. It is evident that a considerably larger number of compounds are resolved by this technique than by HPLC (Fig. 4). In fact the GC method is routinely used for the simultaneous analysis of 22 PAHs and this number could be increased if desired.

Some of the disadvantages of the methods above are already being overcome by improvements in chromatographic technology. Kong *et al.*[12] have described a mesogenic polysiloxane stationary phase which provides good separation of PAH isomers when used for capillary GC. This liquid crystal phase is able to separate the chrysene/triphenylene and benzo[*b*]fluoranthene/benzo[*k*]fluoranthene/benzo[*j*]-fluoranthene isomers quite readily and seems a significant improvement on the SE-52 column with which it was compared. Similarly the advent of the Perkin-Elmer LS5 spectrofluorimeter, which allows changes in excitation/emission monochromator settings at predetermined times during a run, has increased the number of PAHs which can be analysed in a single chromatogram at low concentrations. By replacing the Model 3000 spectrofluorimeter with this instrument it has recently been possible to reduce the detection limits for pyrene (to 0.20 $\mu g\ kg^{-1}$) and benzo[*e*]pyrene (to 0.30 $\mu g\ kg^{-1}$) and to perform conveniently the previously difficult analyses of chrysene and indeno [1,2,3-*c*,*d*]pyrene at detection limits of 0.05 and 0.75 $\mu g\ kg^{-1}$ respectively.

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