ELSEVIER

Journal of Chromatography A, 734 (1996) 297–302

JOURNAL OF CHROMATOGRAPHY A

Determination of monocyclic aromatic hydrocarbons in fruit and vegetables by gas chromatography–mass spectrometry[1]

Anna Górna-Binkul[a], Regine Keymeulen[b], Herman Van Langenhove[b], Bogusław Buszewski[a,*]

[a]*Department of Environmental Chemistry, Faculty of Chemistry, Nicholas Copernicus University, 7 Gagarin St, PL-87 100 Toruń, Poland*

[b]*Department of Organic Chemistry, Faculty of Agricultural and Applied Biological Sciences, University of Ghent, 653 Coupure Links, B-9000 Ghent, Belgium*

First received 14 June 1995; revised manuscript received 3 January 1996; accepted 9 January 1996

Abstract

Monocylic aromatic hydrocarbons (MAHs: benzene, toluene, ehtylbenzene and xylenes) were isolated from fruit and vegetables using a solvent extraction technique. GC–MS (with selected-ion monitoring mode) was applied for determination of the isolated pollutants. It was observed that uptake of MAHs depends on the species and takes place in different morphological parts of the biological material. The highest concentrations of MAHs were found in parsley leaves (*m*- and *p*-xylene) and in orange peel (toluene). Estimation of the daily human exposure to MAHs through eating contaminated fruit and vegetables was performed.

Keywords: Fruits; Vegetables; Food analysis; Benzene; Toluene; Xylenes; Ethylbenzene

1. Introduction

Monocyclic aromatic hydrocarbons (MAHs: benzene, toluene, ethylbenzene and xylenes) are important organic air contaminants, considered by the US Environmental Protection Agency as priority toxic pollutants because of the ubiquity of their emission sources and their harmful effects on people and animals.

MAHs are mainly emitted into the atmosphere by fossil fuel combustion and evaporation (automobile exhaust, thermal power plants, storage, transfer and handling of fuel, refuelling, etc.). Since these pollutants can spread over considerable distances in the atmosphere, they are present in both industrial and rural areas. Measurements of aromatic hydrocarbons in ambient air, sampled in a number of cities around the world, show that average concentrations of MAHs vary from 1 to 70 $\mu g\ m^{-3}$, with the highest for toluene and the lowest for *o*-xylene [1–5].

The greatest health risk from exposure to aromatic hydrocarbons is due to benzene, which is carcinogenic. Exposure to benzene may lead progressively to leukaemia, aplastic anaemia and

[1] Presented at the 7th Symposium on Handling of Environmental and Biological Samples in Chromatography, Lund, Sweden, 8–10 May 1995.

* Corresponding author.

0021-9673/96/$15.00
PII S0021-9673(96)00038-6

dysfunction of the immune system [6]. Potential danger to human health is also caused by toluene, ethylbenzene and xylenes, the toxic properties of which have frequently been studied [7–9]. In addition, MAHs are photochemically reactive, so the intermediate products that are formed in the photochemical cycle can also have toxic effects.

Once released into the atmosphere, MAHs distribute among the environmental compartments. Two of the most important physicochemical properties of MAHs, which determine their environmental fate, are volatility and lipophilicity. The latter property indicates that bioaccumulation of MAHs can take place in lipophilic parts of plants. Absorption of MAHs in edible plants (fruit, vegetables) would be of great importance, since they are used for human nutrition. Up to now, most publications on the sorption of chemicals in edible plants have related to pesticides, and only a few have considered volatile compounds, e.g., Topp et al. [10] reported the uptake of benzene in barley leaves.

The aim of this study was to determine average concentrations of benzene, toluene, ethylbenzene and xylenes in various species of fruits and vegetables in order to evaluate the importance of human exposure to these contaminants via the food chain.

2. Experimental

The method that was developed to determine MAH concentrations in plant leaves [11] was adapted to fruit and vegetables. It consists of solvent extraction of biological material followed by GC–MS analysis of the extracts.

2.1. Sample preparation

Fruit and vegetables (specified in Tables 2 and 3) were purchased in local shops and stalls. Selected parts were cut into small pieces using a knife and placed in glass tubes by using tweezers to avoid losses of volatile compounds by warming of samples during handling.

Dichloromethane (Merck, Darmstadt, Germany), containing an internal standard (perdeuterooctane at 5 pl μl^{-1}), was added to the tubes to extract MAHs from the fruit and vegetables. A 0.5–1.5-ml volume of CH_2Cl_2 was used for each tube, depending on the mass of the material (0.5 ml of extraction solvent per gram of fresh biological material). The tubes were closed with screw-caps and PTFE tape and placed in a slowly rotating drum for at least 6 h (the optimum extraction time found to extract MAHs from plant leaves [11]). The extracts were then filtered (Millex-HV 0.45-μm filter) and the filtrates were collected in small bottles, which were tightly closed again and stored in a freezer. The residual material after filtration was dried in an oven at 50–60°C for 7 days and weighed again to determine the dry mass of the samples.

2.2. Chromatographic investigations and apparatus

Analysis was performed by injecting aliquots of 1 μl of the fruit and vegetable extracts. Also, injections of 1 μl of a stock standard solution, consisting of five reference MAHs at 1 pl μl^{-1} and internal standard at 5 pl μl^{-1} in dichloromethane, were made to obtain data for the calculations of MAH concentrations in the extracts.

An HP 5890 gas chromatograph (Hewlett-Packard, Waldbronn, Germany) coupled with a Model 5970A quadrupole mass spectrometer, and equipped with an HP 200 computer system (for separation process control and data collection), was used for the analyses. The gas chromatograph was provided with a 50 m × 0.258 mm I.D. fused-silica capillary column coated with a 0.25-μm thick layer of DB-5 stationary phase (J&W Scientific, Folsom, CA, USA) connected with splitless injection. The carrier gas was helium at a linear velocity of 0.48 m s^{-1}. The injector temperature was 250°C and the GC–MS interface temperature was 260°C. The initial oven temperature was 28°C, increased at 2°C min^{-1} for 17 min and then at 15°C min^{-1} to 240°C.

The quadrupole mass spectrometer was programmed in the selected-ion monitoring (SIM) mode, which enables optimum results to be

obtained owing to selective detection of the ions characteristic of the compounds of interest. The detailed MS parameters for data acquisition, such as the time interval for each group of ions and characteristic ion masses, are presented in Table 1.

2.3. Qualitative determination

To identify compounds of interest in the fruit and vegetable extracts, retention times and mass spectra of MAHs obtained from GC–MS analysis of the stock standard solution were used. For example, from the comparison, peaks in the chromatograms of orange peel extract (Fig. 1) and parsley leaves extract (Fig. 2) correspond to benzene (retention time 5.484 min), toluene (9.124 min), perdeuterooctane (10.236 and 10.290 min), ethylbenzene (14.468 and 14.623 min), *m*-/*p*-xylene (15.034 and 15.199 min) and *o*-xylene (16.631 and 16.818 min).

2.4. Quantitative determination

Concentrations of MAHs in the extracts were calculated by calibrating the peak area using data from injections of the stock standard solution (three injections for each analysis). The detailed calculation procedure is presented below:

Calibration units of MAH (U):

$$U = (B_S D_E)/D_S \quad (1)$$

where B = peak area of MAH, D = peak area of perdeuterooctane and subscripts S = stock solution and E = extract.

Amount of MAH in the extract (A_E):

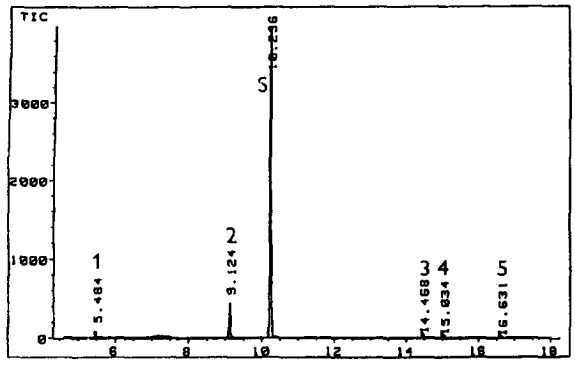

Fig. 1. Chromatogram of extract of orange peel. Peaks: 1 = benzene; 2 = toluene; S = perdeuterooctane (internal standard); 3 = ethylbenzene; 4 = *m*-/*p*-xylene; 5 = *o*-xylene. Time scale in min.

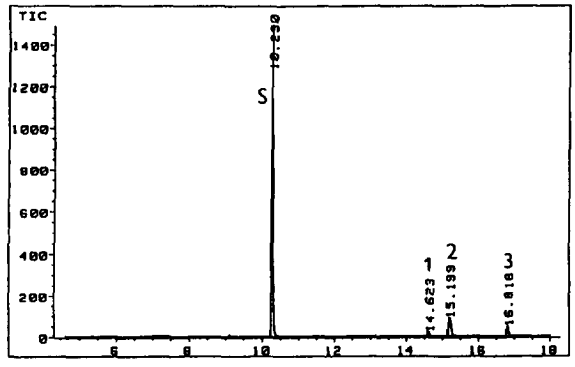

Fig. 2. Chromatogram of extract of parsley leaves. Peaks: S = perdeuterooctane (internal standard); 1 = ethylbenzene; 2 = *m*-/*p*-xylene; 3 = *o*-xylene. Time scale in min.

$$A_E = [(B_E C_S)/U]V\ (\mu g) \quad (2)$$

where C = concentration of MAH ($\mu g\ ml^{-1}$) and V = volume of extraction solvent (ml).

The concentration of MAH in the fruit or

Table 1
Parameters for data acquisition in SIM mode

No. of group	Time interval of sampling (min)	m/z	Compound	Scan rate for sampling (cycles/min)
1	4.50–7.50	77, 78	Benzene	3.7
2	7.50–9.80	91, 92	Toluene	2.0
3	9.80–12.50	66, 98	Perdeuterooctane	2.0
4	12.50–18.00	91, 106	Ethylbenzene, *m*-/*p*-xylene, *o*-xylene	2.0

vegetable ($\mu g\, g^{-1}$ dry mass) is obtained by dividing A_E by the mass of dry biological material.

3. Results and discussion

3.1. Analysis of extracts

The concentrations of benzene, toluene, ethylbenzene, *m*-/*p*-xylene and *o*-xylene in the ten fruit species examined are presented in Table 2. Benzene was found in only three species (apple, kiwifruit and orange) and at low concentrations (27–56 ng g^{-1} dry mass), whereas toluene was detected in eight. It should be noted that toluene was found in all *Citrus* species examined (orange, grapefruit, lemon and mandarin) with the concentrations (169–771 ng g^{-1} dry mass) higher than in the other species (apple, kiwifruit, pear, plum). Xylenes were only detected in the peel extract of orange, but the concentrations were low (between 111 and 3 ng g^{-1} dry mass).

In Table 3 are presented the concentrations of MAHs in the fourteen species of vegetables examined. Benzene was not detected and toluene was found in only a few species (cabbage, tomato, paprika, Brussels sprouts). Xylenes were detected in only two vegetables, parsley and paprika, but the concentrations (up to 1890 ng g^{-1} dry mass) were higher than those of toluene (90–229 ng g^{-1} dry mass).

From the data in Tables 2 and 3, it can be seen that the sorption of MAHs is dependent both on the species and on the morphological part. For example, orange peel was found to absorb all the compounds of interest, whereas in avocado peel and celery leaves none of the MAHs were detected. In addition, for the species for which both extracts (from peel and pulp) were tested, higher toluene concentrations were measured in peel than in pulp. This can be explained by the presence in peel of lipophilic components (wax layer, essential oils), which exhibit a higher uptake of lipophilic compounds such as MAHs. Topp et al. [10] also observed a higher uptake of lipophilic organic compounds (benzene, chlorinated benzenes) in oil-containing plants (effective additional uptake mechanism due to higher lipid content).

Moreover, it should be noted that in none of the extracts of underground parts (roots, bulb) of different vegetable species (rooted turnip, potato, radish, parsnip, celeriac, carrot) were MAHs found. These parts are not directly exposed to polluted ambient air during growth and maybe their contamination did not occur. However, it should be realized that not only are fruit and vegetables exposed to air pollutants during their vegetation (kitchen gardens near high-traffic streets; fields and orchards close to power-stations, metal industries), but also additional contamination can take place during their transport, storage and sale (stands situated near cross-roads, fuel stations, dry cleaners). Since various sources could pollute the experimental material (fruit and vegetables purchased in the shops), it is important to add that we could only measure average concentrations of MAHs, and we were not able to evaluate the contribution of individual contamination sources to the total content of these compounds in the fruit and vegetables.

3.2. Human exposure of MAHs through nutrition

In the assessment of the human health risk caused by exposure to MAHs through nutrition, the concentrations measured in fruit and vegetables (Tables 2 and 3) were used. Assuming daily consumption of the following fruit and vegetables: apple (two pieces), pear, tomato, paprika (one piece each) and cabbage with parsley leaves (salad), exposure to MAHs via the food chain was calculated and the results are shown in Table 4. According to these data, daily exposure to MAHs through consumption of contaminated fruit and vegetables amount to 15.6 μg. However, it should be noted that the exposure may differ significantly from the absorbed dose (intake), since many factors (e.g., water solubility, fat solubility, composition of the food) influence the transportation, distribution and final tissue concentrations of MAHs in the human body. Unfortunately, literature data on the absorption

Table 2
Concentrations of monocyclic aromatic hydrocarbons in fruit ($\mu g\ g^{-1}$ dry mass)

Compound	Apple		Kiwifruit		Pear		Plum		Orange peel	Lemon peel	Grapefruit peel	Mandarin peel	Avocado peel	Grape peel
	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp						
Benzene	0.0266	–	0.0321	–	–	–	–	–	0.0558	–	–	–	–	–
Toluene	0.0879	0.0464	0.0556	0.0350	0.0653	–	0.0498	–	0.7710	0.2506	0.3610	0.1693	–	–
Ethylbenzene	–	–	–	–	–	–	–	–	0.0236	–	–	–	–	–
m-/*p*-Xylene	–	–	–	–	–	–	–	–	0.1108	–	–	–	–	–
o-Xylene	–	–	–	–	–	–	–	–	0.0029	–	–	–	–	–

Table 3
Concentrations of monocyclic aromatic hydrocarbons in vegetables ($\mu g\ g^{-1}$ dry mass)

Compound	Tomato		Paprika peel	Chicory leaves	Cabbage leaves	Brussels sprouts leaves	Rooted turnip root	Parsley leaves	Potato bulb	Radish root	Bean pod	Celery leaves	Parsnip root	Celeriac root	Carrot root
	peel	pulp													
Benzene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Toluene	0.1729	0.1072	0.1313	–	0.2285	0.0899	–	–	–	–	–	–	–	–	–
Ethylbenzene	–	–	–		–	–	–	0.2567	–	–	–	–	–	–	–
m-/*p*-Xylene	–	–	0.2131	–	–	–	–	1.8896	–	–	–	–	–	–	–
o-Xylene	–	–	–	–	–	–	–	0.7458	–	–	–	–	–	–	–

Table 4
Daily human exposure to MAHs originating from consumption of fruit and vegetables

Sample	Fresh mass[a] (g)	Water content[b] (%)	Dry mass (g)	Concentration of MAH[c] ($\mu g\ g^{-1}$ dry mass)					Daily exposure to MAH ($\mu g\ day^{-1}$)				
				B	T	E	*m*-/*p*-X	*o*-X	B	T	E	*m*-/*p*-X	*o*-X
Apple peel	46	74	12.0	0.0266	0.0879	–	–	–	0.3	1.1	–	–	–
Apple pulp	360	83	61.2	–	0.0464	–	–	–	–	2.8	–	–	–
Pear peel	24	77	5.8	–	0.0653	–	–	–	–	0.4	–	–	–
Tomato peel	12	88	1.4	–	0.1729	–	–	–	–	0.2	–	–	–
Tomato pulp	160	95	8.0	–	0.1072	–	–	–	–	0.9	–	–	–
Paprika	160	93	11.2	–	0.1313	–	0.2131	–	–	1.5	–	2.4	–
Cabbage leaves	150	90	15.0	–	0.2285	–	–	–	–	3.4	–	–	–
Parsley leaves	5	83	0.9	–	–	0.2567	1.8896	0.7458	–	–	0.2	1.7	0.7

[a] Approximate mass.
[b] Calculated using fresh and dry mass of examined fruit and vegetable samples.
[c] Concentration of MAHs found in examined fruit, vegetable samples, where B = benzene, T = toluene, E = ethylbenzene, X = xylene.

of MAHs in humans are scarce [12]. Also, no data on the acceptable daily intake of MAHs via fruit and vegetables could be found. According to the results of the DGMK project [13], toluene is completely absorbed from the gastro-intestinal tract after oral dosing, but for benzene no data are available [14]. In addition, has been observed that if toluene is dissolved in vegetable oil, absorption is less efficient [15].

Moreover, in estimating the importance of the dietary uptake of MAHs through nutrition, other kinds of food should also be taken into consideration, since they too could be contaminated. For example, Grob et al. [16] have found that foodstuffs such as butter, oil, bacon fat, cheese absorbed MAHs in considerable amounts. Hence consumption of these foods could contribute to the amount of MAHs to which humans are exposed.

4. Conclusions

From differences in the concentrations of benzene, toluene, ethylbenzene and xylenes in the fruit and vegetables examined, it can be concluded that the sorption of MAHs is dependent both on the species and on the morphological part. In underground parts, i.e., roots and bulbs (not directly exposed to polluted ambient air during growth), of the vegetable species carrot, parsnip, celeriac, potato, rooted turnip and radish no MAHs was detected. A higher toluene uptake in peel than in pulp of apple, kiwifruit and tomato was observed. This was probably due to the high content in the peel of components with affinity to lipophilic compounds, to which MAHs belong.

Further investigations are necessary to find the causes of the differences in the uptake of MAHs in various fruits and vegetables. For instance, experiments on the composition of the peel (e.g., orange peel, since it was found to absorb all compounds of interest) could give some explanation.

Acknowledgement

Anna Górna-Binkul is grateful to TEMPUS (JEP 2237) for financial support.

References

[1] C.W. Sweet and S.J. Vermette, Environ. Sci. Technol., 26 (1992) 165.
[2] B.M. Wathne, Atmos. Environ., 17 (1983) 1713.
[3] H.B. Singh, L.J. Salas, B.K. Cantrell and R.M. Redmond, Atmos. Environ., 19 (1985) 1911.
[4] C. Lanzerstorfer and H. Puxbaum, Water Air Soil Pollut., 51 (1990) 345.
[5] P. Bruckman, W. Kersten, W. Funcke, E. Balfanz, J. Köning, J. Theisen, M. Ball and O. Päpke, Chemosphere, 17 (1988) 2363.
[6] A. Yardley-Jones, D. Anderson and D.V. Parke, B. J. Ind. Med., 48 (1991) 437.
[7] M. Sitting, Toxic Priority Pollutants, Health Impacts and Allowable Limits, Noyes Data, Park Ridge, NJ, 1980.
[8] L. Fishbein, Sci. Total Environ., 42 (1984) 267.
[9] L. Fishbein, Sci. Total Environ., 43 (1985) 165.
[10] E. Topp, I. Scheunert, A. Attar and F. Korte, Ecotoxicol. Environ. Saf., 11 (1986) 219.
[11] R. Keymeulen, H. Van Langenhove and N. Schamp, J. Chromatogr., 541 (1991) 83.
[12] H.J.Th. Bloemen and J. Burn (Editors), Chemistry and Analysis of Volatile Organic Compounds in the Environment, Blackie, Glasgow, 1994.
[13] DGKM Project 174-7, Deutche Gesellschaft für Minerolwissenschaft und Kohlechemie, Hamburg, 1985.
[14] W. Sloof, Integrated Critera Document: Benzene, Report No. 75846001, National Institute of Public Health and Environmental Protection, Bilthoven, 1987.
[15] K. Pyykko, H. Tahti and H. Vapaatalo, Arch. Toxicol., 38 (1977) 169.
[16] K. Grob, C. Frauenfelder and A. Artho, Z. Lebensm. Unters. Forsch., 191 (1990) 435.