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Qualitative TLC determination of some polycyclic aromatic hydrocarbons in sugar-beet

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Abstract: The presence of polycyclic or polynuclear aromatic hydrocarbons (PAHs) were investigated in sugar-beet from a local sugar factory in the district of Vojvodina. The sugar-beet was cultivated on areas near roads with intensive traffic. The procedure for the preparation and determination of these compounds included saponification of the sample, several liquid–liquid extraction systems and a silica gel column clean-up. The purified sample solution was analysed by thin layer chromatography (TLC) on silica gel with cyclohexane as the developing solvent. Benzo(b)fluoranthene and benzo(a)anthracene and/or benzo(a)pyrene were detected at concentrations greater than the allowed limits in food.

Keywords: PAHs, sugar-beet, cyclohexane extracts, TLC, silica gel.

INTRODUCTION

PAHs and their derivatives are emitted during combustion processes and they usually occur (particularly those that are the most dangerous for human health) suspended in the air. These compounds are ubiquitous environmental pollutants that are of concern because of their mutagenicity and/or carcinogenicity.[1–3] Intensive investigations have been carried out on the determination of toxic organic compounds emitted into the atmosphere because of the progressive environment degradation. In plant samples and food, the presence of PAHs is attribute exclusively to environment contamination. Agricultural crop foods may be contaminated by deposition of airborne PAHs, originating from industrial activities, heating and transport. In addition, contaminated soil and water contribute to the increase of the concentration of PAHs on/in mass cultures. Decision No. 2455/2001/EC of the European Parliament and of the Council established a list of priority substances in the field of water policy and amended Directive 2000/60/EC, introduced scientifically based methodology for selecting priority substances on the basis of their significant risk to or *via* the

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aquatic environment. PAHs belong to the category of priority substances in accordance to the above-mentioned Directive.[4] The main source of human intake of PAHs *via* food is by the consumption of cereal products, edible oils and fats. In order to determine the presence of PAHs in food, it is necessary to isolate them from a very complex matrix.

TLC has been used for the determination and identification of PAHs and their derivatives,[3,5–10] as well as of many other compounds which contaminate air, water, food, sludge and other elements of the natural environment.[9] The purpose of the present study was the determination of PAHs in sugar-beet using a procedure which involve extraction and concentration of the PAH fraction and analyse of the extract by TLC.

EXPERIMENTAL

Chemicals

All chemicals used were of analytical-reagent grade: methanol, cyclohexane, acetone and dimethylformamide (DMF) and column silica gel 60 (70–230 mesh) were obtained from Merck (Germany), potassium hydroxide and sodium sulphate from Zorka-Pharm (Serbia and Montenegro)

Standards

PAH standard substances were commercial products (with purities of 95 – 99 %): benzo(a)anthracene, BaA, and benzo(b)fluoranthene, BbF, (Supelco), benzo(a)pyrene, BaP, (Fluka), anthracene, A and phenanthrene, Ph (Merck).

PAH standards solutions

0.6 μg BaA/ml, 18.75 μg BbF/ml, 210 μg A/ml, 380 μg Ph/ml and 51.5 μg BaP/ml. The solutions of the standard substances were prepared in acetone and stored at 4 ºC in the dark.

Sample

A composite sample, obtained by mixing individual sugar-beets, was collected from a local sugar factory in the region of South Bačka (Žabalj, Vojvodina, Serbia and Montenegro) during processing in the autumn of 2002. The sugar-beet had been cultivated in areas near the factory. The sample, *i.e.*, sugar-beet pulp, was prepared in duplicate by milling the composite sample.

Extraction

A mass of 151 g of sugar-beet pulp with 24.63 % dry matter was digested in 220 ml of a methanolic solution (methanol/water = 9 + 1) of potassium hydroxide, c(KOH) = 2 mol/dm^3. The further procedure followed exactly the method described by Larsson,[11] with the exception that the first 23 ml of the eluate from a silica gel column was also collected and analyzed (Fraction I), as was 23–100 ml of the eluate (PAH fraction, Fraction II).

The analytical scheme for the isolation and analysis of the PAHs from sugar-beet is presented in Fig. 1.

The extracts of the sample were concentrated to about 500 μl under a stream of nitrogen in the dark.

Spike probe

Volumes of 300 μl BaA and 11 μl BbF were added to 75 ml of a methanolic solution (methanol/water = 9 + 1) of potassium hydroxide, c(KOH) = 2 mol/dm^3. The further procedure was the same as described above for the sample.

TLC analysis

TLC was performed on (20 × 20 cm) commercial plates precoated with silica gel G with a fluorescence indicator (Merck) and on thin-layers of aluminum oxide G, Type E (Merck) prepared in our laboratory. The aluminum oxide (30 g) was suspended in 40 ml of distilled water and 0.2 % of fluorescence indicator F_{254}

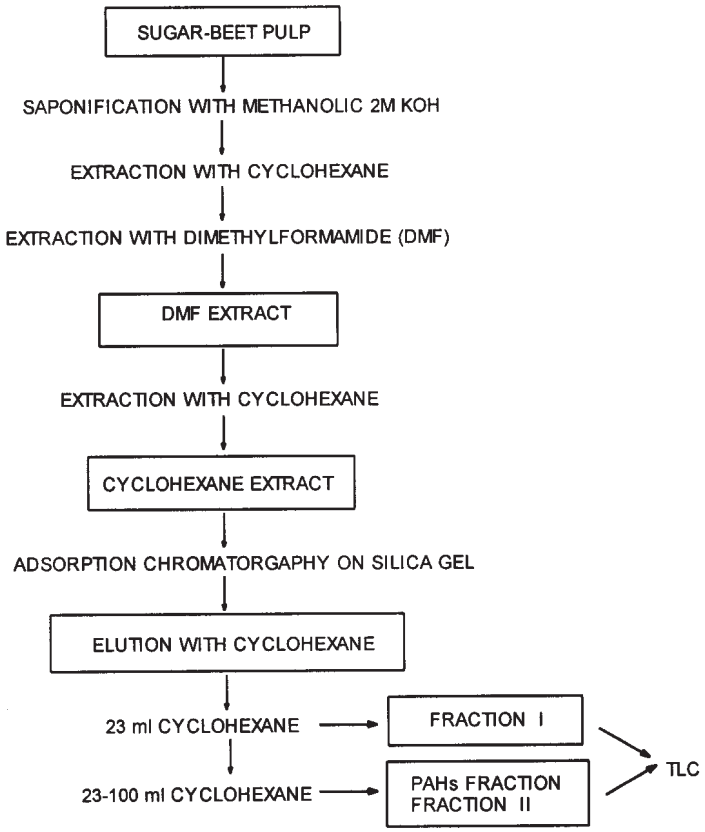

Fig. 1. Analytical scheme for the isolation and analysis of PAHs in sugar-beet.

(Merck) was added to the suspension. The suspension was coated onto glass plates with Desaga equipment and the layers were dried in air at room temperature. All the plates were activated by a standard method before use: drying at 120 ºC for 1 h.

The PAH standard soltuion (1–100 μl), spike probe and sugar-beet extracts (10–30 μl) were spotted onto the chromatographic plates. The chromatograms were developed in the dark using *n*-hexane–benzene (1:1, v/v), ethyl acetate, *n*-hexane and cyclohexane as the mobile phases.

The chromatograms were observed under illumination at λ = 254 and 365 nm.

RESULTS AND DISCUSSION

In this study, TLC was applied for the identification and determination of some PAHs in sugar-beet from a local sugar factory. The standards were five PAH compounds from the U.S. EPA list with different numbers of aromatic rings and different degrees of condensation. The results of the TLC analyses of the PAH standard substances are listed in Tables I and II. The R_f values of the PAH standards on silica gel and aluminum oxide layers using different developing systems are presented in Table I.

It is apparent from Table I that the PAH standards substances have similar R_f values on silica gel and aluminum oxide and cannot be separated, irrespective of the mobile phase employed: BbF, BaP and BaA (R_f = 0.35 – 0.42), A and Ph (R_f = 0.70 – 0.78). Silica gel

with cyclohexane as the mobile phase were selected for further identification of the PAH fractions. The range of R_f values, fluorescence colours at 254 and 365 nm and the detection limits of the PAH standards on silica gel with cyclohexane as the developing system are listed in Table II.

TABLE I. R_f Values of the PAH standards on silica gel and aluminum oxide using different developing systems

Compounds	Abbreviation	R_f							
		n-Hx-Bz (1 : 1)	EtAc	*n*-Hx	CyHx	*n*-Hx-Bz (1 : 1)	EtAc	*n*-Hx	CyHx
		Silica gel				Aluminum oxide			
Benzo(b)fluoranthene	BbF	0.41	0.33	0.35	0.37	0.39	0.33	0.39	0.39
Benzo(a)pyrene	BaP	0.39	0.35	0.40	0.40	0.39	0.33	0.35	0.40
Benzo(a)anthracene	BaA	0.42	0.35	0.41	0.41	0.43	0.34	0.37	0.42
Anthracene	A	0.78	0.75	0.73	0.74	0.74	0.73	0.70	0.73
Phenanthrene	Ph	0.78	0.75	0.75	0.76	0.74	0.73	0.70	0.73

Hx–Hexane; Bz–benzene; EtAd–ethyl acetate; CyHx–cyclohexane

TABLE II. Range of R_f values, spot colours and detection limits of the PAHs standards developed on silica gel with cyclohexane

Compound*	Range of R_f	Spot colour		Detection limit/ng
		λ = 254 nm	λ = 365 nm	
BbF	0.35–0.37	Blue	Yellow	18.75
BaP**	0.38–0.42	Violet	Pink-violet	276
BaA	0.40–0.42	Blue	Light blue	60
A	0.68–0.75	Blue	Pink	210
Ph	0.66–0.76	Dark violet	Pink-violet	380

*The abbreviations are explained in Table I; **BaP is yellowish in visible light

Low detection limits were obtained for BbF and BaA, *i.e.*, 18.75 and 60 ng, respectively. However, the detection limits of the other standards were much higher, *i.e.*, 210–515 ng (Table II).

Fractions, I and II (Fig. 1) from the spike probe and sugar-beet sample were analyzed chromatographicaly. The chromatograms were developed for about 130 minutes to a distance of 15 cm.

The spike probe confirmed the efficiency of the Larsson extraction procedure for PAHs.[1,11] BbF and BaA were identified only in fraction II, *i.e.*, the PAH fraction.

The results of the TLC analyses of sugar-beet cyclohexane extracts indicate a complex composition of the fractions tested. Fraction I, *i.e.*, the first 23 ml of the cyclohexane extracts, contained some unidentified non-polar compounds with R_f values above 0.6. On the chromatograms of fraction II, nine spots were observed with R_f values in the range 0.1–1.0. By comparison with the R_f values of the standard substances, as well as of the flu-

orescence and colour presented in Table II, BbF (R_f = 0.37) and BaA and/or BaP (R_f = 0.42) were identified in the analyzed sample of sugar-beet. According to detection limits of BbF, BaA and BaP (Table II), the intensity of the illumination of the spots (especially when observed at λ = 365 nm) and the volumes of the sample extract spotted onto the chromatographic plate (30 μl), it is evident the presence of these compounds at levels higher than 8 ng (BbF), 27 ng (BaA) and 124 ng (BaP) calculated on 1 g of dry sample was confirmed by TLC analyses. These concentrations are greater than the limits allowed in food: 0.1; 0.1 and 0.3 ng/g for BbF, BaA and BaP, respectively.[12] Probably, the observed, rather high amounts of PAHs found is closely connected with the region of the sugar-beet cultivation. The whole region is located near highways with intensive traffic and approximately 20 km away from Novi Sad, a center of commercial and industrial activities.

The chromatographic characteristics of BaA and BaP (Table II) indicate that it is impossible to identify their presence individually. For certain identification, the fraction BaA/BaP should be further investigated by gas chromatography-mass spectrometry (GC-MS).[5]

The determination of the content BaP and BaA is extremely important and has been intensively studied because of their highly carcinogenic and toxic features.[1,11,13,14] Benzo(a)pyrene, benzo(a)anthracene and benzo(b)fluoranthene, together with fluoranthene, benzo(k)fluoranthene, indeno(1,2,3,c,d)-pyrene and benzo(g,h,i)perylene, are the most toxic PAHs on the National Bureau of Standard List.[15] The Department of Health and Human Services (DHSS) has determined that benzo(a)anthracene, benzo(b)fluoranthene, benzo(a)pyrene and others are known animal carcinogens. This is important because sugar-beet may be used as animal fodder. According to the International Agency for Research on Cancer (IARC), bennzo(a)anthracene and benzo(a)pyrene are probably carcinogenic to humans and according to EPA, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene and a few others are probable human carcinogens.[16]

Classical adsorption TLC is a simple and quick analytical method, however, because of the high detection limits and similar R_f values of some of the PAHs, it is not useful for their unambiguous identification in a sample such as sugar-beet. Nevertheless, application of TLC is possible as a preliminary method, especially for more contaminated samples such as soils, sewage sludges and airborne particulate matter near the emission sources.[5,8]

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Abbreviations:

A = Anthracene
BaA = Benzo(a)anthracene
BaP = Benzo(a)pyrene
BbF = Benzo(b)fluoranthene
Bz = Benzene

CyHx = Cyclohexane
DMF = Dimethylformamide
EtAc = Ethyl acetate
Hx = Hexane
PAH = Polycyclic or polynuclear aromatic hydrocarbon
Ph = Phenanthrene
Sample = Sugar-beet
TLC = Thin layer chromatography
U.S. EPA = United States Environmental Protection Agency

ИЗВОД

КВАЛИТАТИВНО ОДРЕЂИВАЊЕ НЕКИХ ПОЛИЦИКЛИЧНИХ АРОМАТИЧНИХ УГЉОВОДОНИКА У ШЕЋЕРНОЈ РЕПИ ХРОМАТОГРАФИЈОМ НА ТАНКОМ СЛОЈУ

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Испитивано је присуство полицикличних ароматичних угљоводоника у шећерној репи из локалне шећеране на подручју АП Војводине. Шећерна репа је гајена на површинама близу путева са интензивним саобраћајем. Поступак за припрему и одређвиање ових једињења укључује сапонификацију узорка, неколико екстракција течно–течно и пречишћавање на колони силика гела. Пречишћен узорак је анализиран хроматографијом на танком слоју силика гела са циклохексаном као мобилном фазом. У узорку шећерне репе је утврђено присуство бензо(б)флуорантена и бензо(а)антрацена и/или бензо(а)пирена изнад граница детекције у храни.

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