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Original Research

Analysis of Chosen Organic Tobacco Smoke Components and Their Metabolites by Planar Chromatography

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Abstract

Planar chromatography was used for one of the final steps of multistage analysis of chosen organic compounds (polycyclic aromatic compounds, nicotine and their derivatives) occurring in tobacco smoke as well as their transformation products. These investigations present various development and detection modes, as well as the possibility of new stationary phase C_{30} application.

The application of planar chromatography has enabled the identification and quantification of many organic pollutants dangerous for human health and the assessment of chosen people groups exposed to tobacco smoke living in the region of Upper Silesia in Poland.

Keywords: planar chromatography, densitometry, environmental samples, body fluids, tobacco smoke exposure

Introduction

Planar chromatography has been popular in many countries, especially for the screening and identification of many substances, but less popular for quantitation [1].

Planar chromatography is employed in analysis, including theoretical as well as practical aspects of chromatographic separations.

This technique enables parallel separation and direct comparison of standards and sample components by the use of various chromatographic systems, as well as development and detection modes. Thin layer chromatography (TLC) does not require special clean-up techniques before the application onto chromatographic plates and provides determination of semi-volatile substances. The possibility of sample component decomposition and difficulty of highly volatile compound analysis, as well as the range of determined compounds is limited – these are the main disadvantages of planar chromatography [1-3].

Planar chromatography has been used to analyze many xenobiotics dangerous for human health, particularly polycyclic aromatic hydrocarbons (PAHs) and their derivatives; some of them can be determined as biomarkers of environmental pollutants. The determination of these compounds is proceeded by multistage analytical procedures, using liquid-liquid and liquid-solid extraction with various modifications [4].

The purpose of this paper was to show various implementation possibilities of the use of planar chromatography for environmental analysis.

In our investigations, planar chromatography has been used mainly to identify and quantify toxic organic pollutants in the environment, where the content of mutagenic and carcinogenic PAHs and their numerous nitrogen and oxygen derivatives more dangerous for human health were determined [5-9]. Some of them also occur in tobacco smoke together with nicotine and other substances from

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the group of tobacco smoke's 4000 ingredients. Many methods have been applied during recent years to analyze nicotine and its metabolites [10-13], among them planar chromatography.

Moreover, tobacco smoke exposure of chosen groups of people from this region was assessed using thin layer chromatography with densitometry for the determination of nicotine and its more durable main metabolites, among them cotinine and *trans*-3'-hydroxycotinine in body fluids.

Experimental procedures

Chemicals

Standards: S-(-)-nicotine, and its metabolites (*trans*-3'-hydroxycotinine, S-(-)-cotinine) were obtained from Toronto Research Chemicals (Ontario, Canada); PAHs and their derivatives were obtained from Fluka (Buchs SG, Switzerland), Sigma (St. Louis, USA), Aldrich (Milwaukee, USA) and Supelco (Bellefonte, Pennsylvania, USA). Solvents were analytical grade, for spectroscopy or for HPLC and delivered by Baker (Griesheim, Germany), Fluka and Merck (Darmstadt, Germany).

Other chemicals were delivered from local commercial sources (POCh, Gliwice, Poland).

Sampling of Body Fluids

Body fluids were sampled from children (576; 10-12 years old), pregnant women (100 – in the second and third semester of pregnancies; the samples were taken up in the Department of Obstetrics and Gynaecology of Medical University of Silesia in Bytom, Poland) and healthy men (47-20 non-smokers; average age of 35 years old and 27 smokers; average age of 33 years old) living in the Upper Silesia region, as well as from first-year students of medicine in the Medical University of Silesia in Zabrze, Poland (89). Sampled biological materials were frozen at –30°C until analysis. Nicotine and its metabolites were isolated from urine [13], amniotic fluid [14] and serum samples [15] by the solid-liquid and liquid-liquid extraction procedures.

TLC

Analysis of Nicotine and Its Metabolites

The recoveries of cotinine from body fluids with various extraction techniques were assessed by planar chromatography [16]. Standards (0.5µg/µl in acetonitrile) and dichloromethane sample solutions were applied (1.5µl –3µl from 20µl) into C_{18}-TLC plates (Machery-Nagel, Düren, Germany) as well as C_{30}-TLC plates (obtained using a procedure described by means of a Nanomat applicator (Camag,Muttenz, Switzerland) [17]). Chromatograms were developed to a distance of 7.5 cm in a horizontal chamber (DSII-Chromdes, Lublin, Poland) using acetonitrile-water (88+12,v/v) with the addition of sodium 1-octanesulfonate (50mg/100ml) as mobile phase. Firstly, visualization was carried out under UV illumination at λ=254nm. Next the spots were quantified by scanning in reflectance mode (at λ=260nm, zigzag scanning mode) using a CS 9301 PC scanner (Shimadzu, Japan). The calibration range was from 50 ng to 200ng/µl of each standard. Identification of nicotine was carried out after densitometric analysis by derivatization with Dragendorff's reagent [18].

PAH Analysis and Their Derivatives

PAHs and their derivatives were chromatographed on silica gel plates with different polarity mobile phase, depending on the analyzed fraction isolated from environmental samples [2].

Standards of hydroxy-PAH were dissolved in acetonitrile and 1 µl of solutions were applied on the C_{18}-TLC plates. The chromatograms were developed with acetonitrile-water (3+2,v/v) and methanol-water (3+1, v/v) in a horizontal DS-chamber and over a pressurized chamber (Cobrabid, Warsaw, Poland) [9]. The standards were scanned by the use of a densitometer in fluorescence mode.

GC-MS

The identification of chosen compounds in investigated samples was also carried out by GC-MS with a GC-14A chromatograph and QP-2000 mass spectrometer (Shimadzu,Japan) under the conditions earlier described [18].

Results and Discussion

Thin layer chromatography was applied for the preliminary investigations as well as for the determination of chosen organic pollutants and their conversion products.

The separation of these compounds can be provided in a horizontal or over a pressurized chromatographic chamber in various modifications (produced in Poland). The semipreparative separation is also possible for thin-layer band chromatography by using a horizontal chamber with a distributor of eluent [3].

Detection and determination of some organic compounds by TLC with densitometry technique depend on numerous agents. First of all, scanning mode, stage and beam, as well as wavelength have the greatest influence on detection limits. Some organic compounds are detected easily or they can be determined only after derivatization.

Densitometric quantitative measurements have been applied for many years for either fluorescence or ultraviolet absorption measurements, while recently the reflection analysis mode for both types has been the most commonly applied [2].

The application of the fluorescence scanning mode very often allows increase of sensitivity and selectivity determinations but fluorescence measurements for many compounds depend strongly on time.

In our previous investigations we tried to determine 1-hydroxypyrene as an ETS (environmental tobacco smoke) biomarker. We have investigated fluorescence dependence on the time of chosen oxidised derivatives polycyclic aromatic hydrocarbons (PAHs), among them OH-PAHs after their separation in reversed phase chromatographic system. Hydroxy-PAHs can be determined by the use of the fluorescence scanning mode, but each of these compounds should be determined in a different time with the highest sensitivity and with maximum fluorescence abundant (Fig 3.). It could be inconvenient in comparison to other methods of PAH metabolite determination.

PAHs exposure can be assessed by the quantification of benzo[a]pyrene DNA-adducts in leukocytes using im-

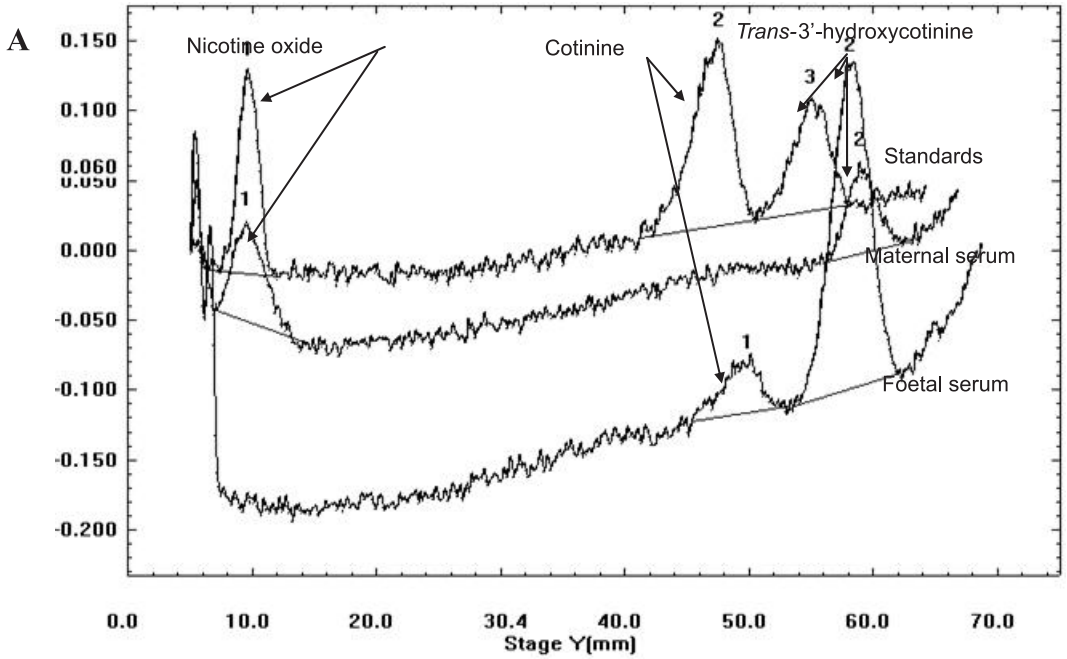

Fig. 1. Densitogram of maternal and foetal serum extracts, in which nicotine metabolites were determined.

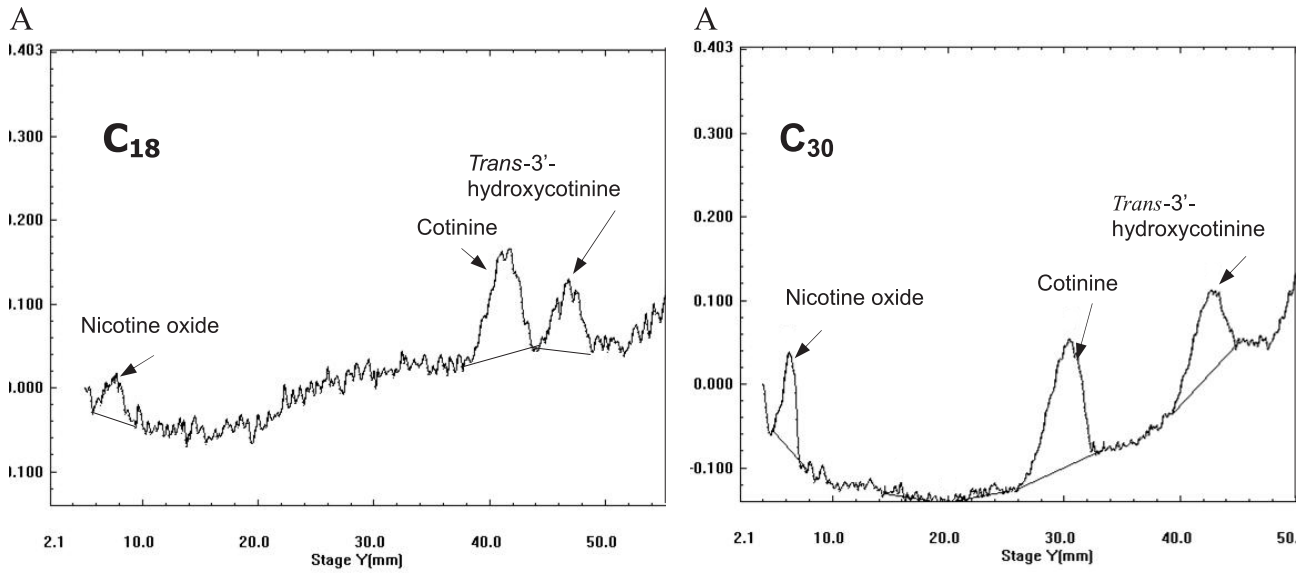

Fig. 2. Densitograms of the main nicotine metabolites separated on C_{18} and C_{30} stationary phases with acetonitrile-water (88+12, v/v) as mobile phase.

munoassays [19], as well as 1-hydroxypyrene in urine by HPLC is more often applied [20].

Some of environmental pollutants (PAH derivatives) can be formed by reactions in the atmosphere or may be the biodegradation products in sewage sludges. They also were identified by planar chromatography [5, 8].

TLC was applied for the preliminary investigations as well as for the determination of main nicotine metabolites in body fluids, using C_{18} TLC plates and zigzag scanning mode.

The worked out procedure of the determination of nicotine and its metabolites were utilized for the assessment of children exposed to tobacco smoke by TLC with densitometry [13]. The range of cotinine concentration in our investigated samples was to 214.6 ng/ml of urine, whereas determination limit was of 1.35ng/ml in urine samples. Intra-day precision was 9.2% for urine, 16.43% for serum, 14.4% for amniotic fluid samples, respectively, in our methods applied for main nicotine metabolites determination. But inter-day precision was not higher than 10.3%. Cotinine recovery from urine, serum and amniotic fluid was 80%, 93.79 and 88%, respectively.

According to our results in approximately 58% of cases we had not stated urinary cotinine above determination limit in comparison to over 53% of Turkish children exposed to ETS [21]. This procedure was also applied for investigations of tobacco smoke influence on asthma in Silesian children [22].

Similar to the results obtained by Johnson and co-workers [23] concerning profiles of the adolescent smoker, more than half of the investigated population of first year medical students (89) have been exposed to tobacco smoke, many of them (according to their reports) exposed to passive smoking from birth. Urinary cotinine concentration in these samples ranged up to 325.9ng/ml.

According to investigations performed by Florek and co-workers, as many as 35.6% of women smoke during pregnancy [24], which was not in agreement with data obtained by questionnaires. We observed similar incompatibility between results of cotinine determination in body fluids and data obtained from interviewed pregnancies [25].

The concentration of cotinine determined in maternal and foetal serum is well correlated (R=0.9057) and was up to 359 ng/ml and 425 ng/ml, respectively. TLC enabled the observation of the differences between the main nicotine metabolites dominating in maternal and foetal serum (Fig.1). Observation that higher cotinine levels were found in fetal than in maternal serum was concluded by Jauniaux and co-workers [26].

In the investigated amniotic fluid samples (50), cotinine was determined in 17 samples, and its concentration was up to 610 ng/ml [14]. This could be a reason for insufficient maturity of newborn babies, as well as their various adolescent disturbances.

The progress of TLC imposes the demand of new stationary phases or modification of those known earlier [17]. For example, recently, C_{30}- TLC plates have been applied to separate the main nicotine metabolite: cotinine and *trans*-3'-hydroxycotinine, and a better separation result has been observed in comparison to C_{18}, the previously used stationary phase. The results of these preliminary investigations are shown in Fig.2. It could also be used in the assessment of tobacco smoke exposure.

In addition, the investigation of nicotine transformation products in air was carried out by planar chromatography using Dragendorff's reagent (Fig.4), which enabled the detection of low amounts of nicotine (10ng/spot). This

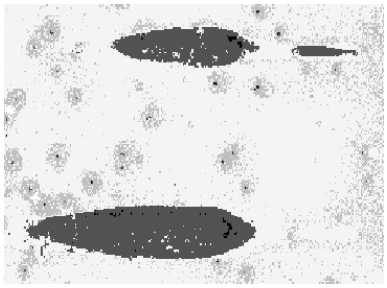

Fig. 3. Dependence of fluorescence intensity on time for 1-hydroxypyrene-A and 2.7-dihydroxynaphthalene-B, measured by densitometer at λ=346 nm

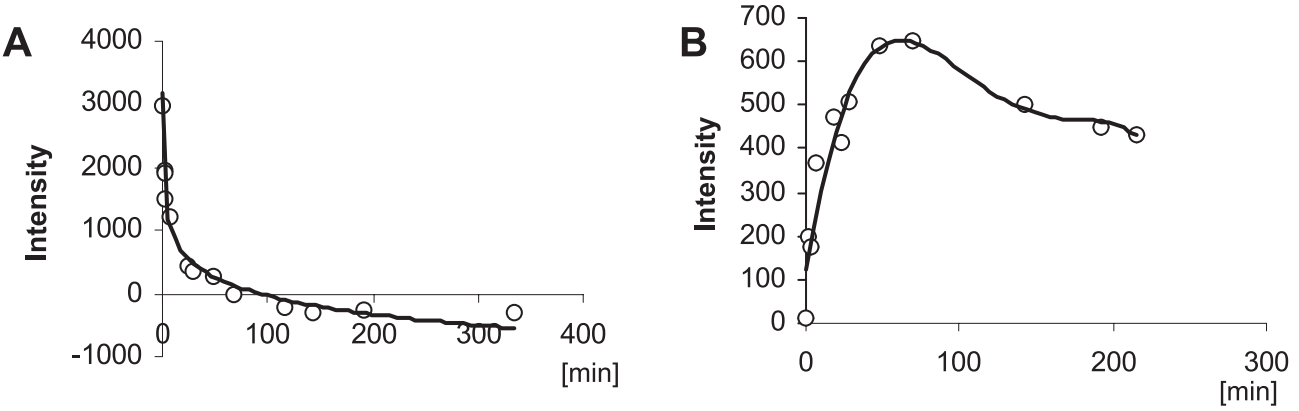

Fig. 4. Nicotine and its transformation products in air after 1 day environmental exposure, derivatised by Dragendorff's reagent (orange spots in yellow background) on the RP-18 plate developed with acetonitrile-water (88+12, v/v).

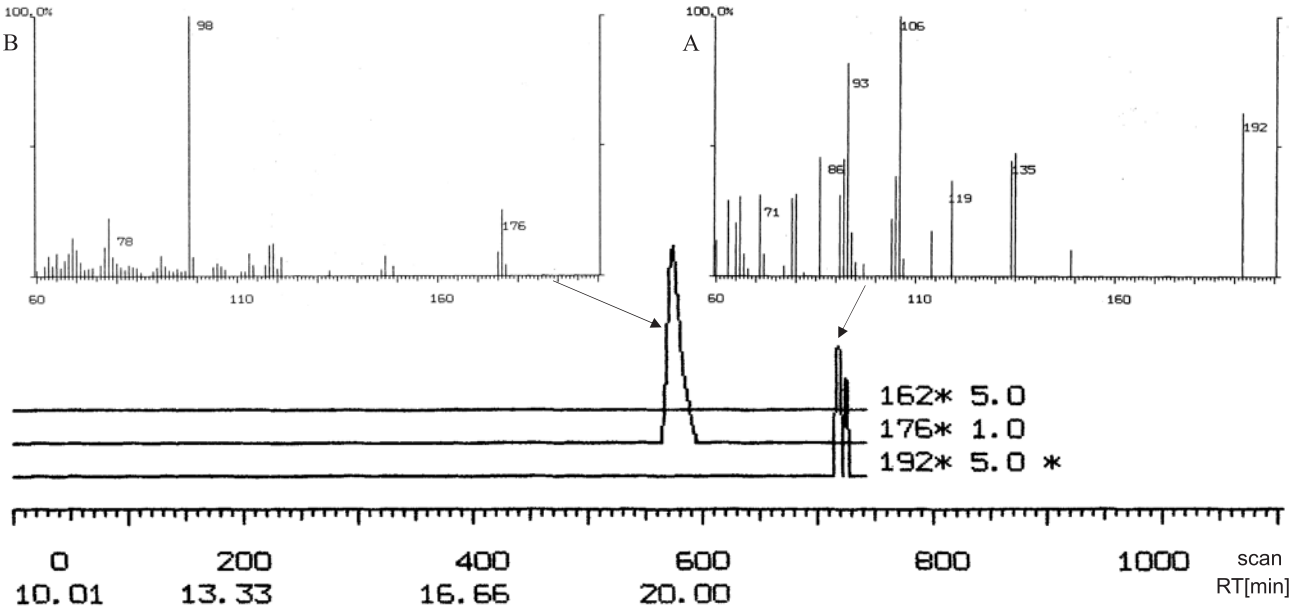

Fig. 5. GC-MS chromatogram and mass spectra of A – cotinine and B – *trans*-3'-hydroxycotinine identified in amniotic fluid.

reaction can also be used to detect unmetabolized nicotine in biological samples [18].

Determination of only these chosen organic compounds, which can occur in living organisms and are responsible for many dangerous diseases, requires the working out of optimization of the chromatographic system but also individual detection and determination conditions after their isolation from environmental samples or body fluids.

In chosen cases the planar chromatography results of analysis were compared with the data obtained by GC-MS technique. It was performed directly in the isolated extracts (e.g. from body fluids) as well as by scraping of analyzed compounds after their separation from TLC plates (nicotine conversion products). Fig. 5 illustrates the confirmation of the main nicotine metabolites present in amniotic fluid.

However, Benowitz [26] and other researches proved that the rate of nicotine metabolism is a determinant of the level of cigarette consumption and metabolism products depend on which kind of metabolizers (rapid or slow) they are. According to Desai and Amin [28] trans-3'-hydroxycotinine is the major urinary metabolite of nicotine in smokers, whereas other authors argue that cotinine should be determined for tobacco smoke exposure [29-31]. That is why chromatographic methods, among them planar chromatography, can be useful in biomarkers determination.

Conclusions

1. TLC with densitometry may not only be a useful technique in the determination of toxic substances in the environment, but also in the biological monitoring of substances dangerous for human health.
2. Analysis of chosen organic pollutants and their metabolites by means of TLC/densitometry was carried out for a short period of time and at minimum toxic solvent consumption.
3. Planar chromatography with densitometry is a simple and economical method to determine biomarkers of tobacco smoke exposure in body fluids. This technique can be used to verify the data on smoking status collected from those interviewed.
4. The application of fluorescence measurement for quantitative analysis of organic compounds requires carrying out of the investigation of fluorescence dependence on time for each of the determined compounds, separately.
5. TLC with densitometry can also be used for the investigation of easily decomposable standard purity.

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