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Fast separation and detection of main components in complex raw biological materials using temperature-controlled planar micro-chromatography (micro-TLC)

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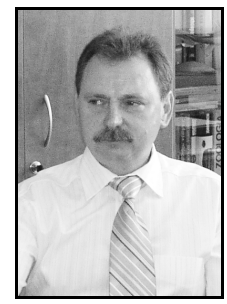

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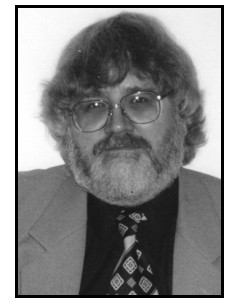

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Abstract

This paper is a continuation of our previous research focusing on development of micro-TLC methodology. The main goal of present paper is to demonstrate the separation and detection capability of micro-TLC technique involving simple analytical protocols without multi-steps sample pre-purification. In present studies components of interest were isolated from biological samples including fish bile and spirulina cells. Described methodology can be applied for fast fractionation or screening of target substances as well as chemo-taxonomic studies and fingerprinting of complex mixtures, which are present in raw biological or environmental samples.

Keywords: planar chromatography, micro-TLC, lyophilization, fish bile, sea trout (Salmo trutta m. trutta), bile acids, spirulina, pharmaceutical formulations, fluorimetric detection, phosphomolybdic acid, temperature, fractionation, fingerprinting.

Szybkie rozdzielanie i detekcja głównych składników złożonych materiałów biologicznych za pomocą mikrochromatografii planarnej (micro-TLC) prowadzonej w warunkach kontrolowanej temperatury

Streszczenie

Chromatografia cienkowarstwowa jest powszechnie wykorzystywaną metodą analityczną stosowaną w rozdzielaniu substancji obecnych w złożonych próbkach biologicznych, środowiskowych oraz preparatach farmaceutycznych. Związane jest to głównie z prostotą sprzętu, możliwością jednoczesnej analizy wielu próbek w trakcie jednego procesu analitycznego. Dodatkowo, istotna jest możliwość bezpośredniego analizowania złożonych próbek bez ich wstępnego oczyszczenia. Obecna publikacja jest kontynuacją badań dotyczących zastosowania mikrochromatografii planarnej prowadzonej w warunkach kontrolowanej temperatury (rys. 1). W szczególności, w pracy wykazano potencjał analityczny mikrochromatografii planarnej w rozdzielaniu złożonych próbek, bez potrzeby ich uprzedniego wieloetapowego oczyszczania. Jako materiały badane wykorzystano spirulinę oraz żółć rybią (rys. 2 - 4). Wykazano, iż detekcja analitów może być znacząco poprawiona poprzez umieszczenie uprzednio rozwiniętych płytek chromatograficznych w parach jodu lub poprzez wybarwienie plamek odczynnikiem wywołującym (kwasem fosforomolibdenowym). Niskocząsteczkowe substancje z próbek środowiskowych (wody z jezior, ścieki surowe oraz oczyszczone) były zatężane przy pomocy ekstrakcji do fazy stałej. Badania wykazały obecność szeregu pasm na mikrochromatogramach cienkowarstwowych, które są specyficzne dla poszczególnych ekosystemów wodnych Pomorza Środkowego. Opisana metodologia może znaleźć zastosowanie w szybkim frakcjonowaniu oraz oznaczaniu ilościowym substancji niskocząsteczkowych pochodzących ze złożonych materiałów biologicznych, jak również w badaniach przesiewowych dużej ilości próbek środowiskowych (rys. 5 - 8).

Słowa kluczowe: chromatografia planarna, mikro-TLC, liofilizacja, żółć ryb, troć wędrowna (Salmo trutta m. trutta), kwasy żółciowe, spirulina, preparaty farmaceutycze, detekcja fluorymetryczna, kwas fosforomolibdenowy, temperatura, frakcjonowanie, fingerprinting.

1. Introduction

In analytical practice thin-layer chromatography (TLC) is still commonly applied method for fast qualitative and quantitative analysis as well as screening of low molecular mass compounds from complex biological and environmental samples [1]. This is mainly due to inexpensive equipment needed and parallel sample processing without pre-purification of the raw materials. Moreover, the advantage of such chromatographic approach is simple detection of separated bands under visible light as well as by using sensitive visualization reagents for a variety of ultraviolet-visible (UV/Vis) transparent bioactive analytes [2]. The resulting spots pattern on developed TLC plate can be easily digitalized using simple office scanners or digital cameras working with dynamic signal range up to 16 bits per RGB

channel. To extend the separation power of the classical TLC plate a high-performance thin-layer chromatography (HPTLC) and/or two-dimensional developing mode can be easily selected. In practice typical separation power of non-forced flow rate HPTLC systems lies between 10 and 20 spots per plate measured in one direction. However, working under 2D-TLC mode the number of spots separated can significantly increase even one factor more [3]. Our experimental data have revealed that 2D developing protocol involving micro-HPTLC plate is capable to separate of more than 240 spots consisting of low molecular mass compounds like steroids or colored substances from cyanobacterial organisms [4, 5].

Noteworthy, under proper experimental conditions, in which the heat evolved during solvent adsorption and mobile phase "distillation process" is minimized, micro-planar chromatographic systems can be very suitable for separation at elevated and sub-ambient temperatures [6]. This is mainly because of the low flow rate and small amount of the mobile phase that is necessarily to perform the separation process. Moreover, there is no Joule's heat evolved due to electric current flow as in *e.g.* planar electrophoresis systems, therefore, new micro-TLC devices, chamber units and microfluid paper-based devices are still constructed [7, 8]. Depends on the plate size, solvent viscosity and temperature, the results of HPTLC separation can be obtained within short period of time even less than 5 min. Recently, number of new detection methods like direct analysis in real time (DART) involving mass spectrometry (MS) techniques was introduced [9, 10]. Such MS-based sophisticated analytical tools including matrix-assisted laser desorption/ionization mass spectrometry (TLC–MALDI-MS) or electron impact ionization mass spectrometry (TLC–EI-MS) were successfully applied for analysis of complex biological samples allowing the use of planar chromatography in metabolomic studies [11].

This paper is a continuation of our previous research focusing on development of micro-TLC methodology [4-7]. The main goal of present paper is to demonstrate the separation and detection capability of micro-TLC technique involving simple analytical protocols without multi-steps sample pre-purification. Components of interest from biological samples including fish bile and spirulina cells were isolated using single pre-treatment steps like direct organic liquid extraction and/or deproteinization by freeze-drying method. Low-molecular-mass compounds from the environmental samples (lake water, untreated and treated sewage waters) were concentrated using optimized solid-phase extraction (SPE). Described methodology can be applied for fast fractionation of target substance and screening as well as fingerprinting of complex mixtures, which are present in raw biological or environmental samples.

2. Experimental

2.1. Materials and reagents

Methanol (LiChrosolv 99.8% for liquid chromatography) was obtained from Merck, Darmstadt, Germany. Acetone (99.9% HPLC grade) was received from Sigma-Aldrich, Steinheim, Germany and n-hexane 95% was a product of Fluka Chemie AG, Buchs, Switrzerland. Double-distilled tap water was used for mobile-phase preparation. Phosphomolybdic acid was purchased from Chempur, Piekary Śląskie, Poland and iodine (cryst., ACS, pure P.A.) was a product of POCH SA, Gliwice, Poland.

2.2. Micro-chromatography and chromatograms digitalization

Separation experiments were performed on glass-based HPTLC RP18W and HPTLC RP18WF_{254}S plates that were products of Merck (Darmstadt, Germany). Before sample application, the factory-prepared plates (100 × 100 mm) were cut to a working size of 50 × 50 mm. In each case, a sample starting line was placed 5 mm from the plate bottom edge, allowing a maximum eluent front migration distance of 45 mm. Micro-planar separations were performed using a home-made temperature-controlled removable horizontal micro-TLC chamber unit, described previously (Fig. 1) [7]. Particularly, a chromium-coated brass unit was working inside a foam insulated metal oven connected to an external liquid circulating thermostat (Ultra-Low Refrigerated Circulator FP51-SL, Julabo, Seelbach, Germany) filled with ethanol as a circulating liquid. The system provided a constant TLC plate temperature, which was set at 30°C (spirulina extracts) or 50°C (sea trout bile samples) with an accuracy of ± 0.02°C. To obtain chromatograms, the following chamber working protocol was applied: a TLC plate with samples spotted on the starting line was positioned horizontally inside a chamber module with the stationary phase layer placed up side down. Afterward, the chamber module was transferred into a thermostating oven cavity and sealed using a 1 mm thin glass cover. Then, the movable cover of the oven was slid so as to reach the position above the TLC chamber module and the temperature equilibration step was performed for 15 min. The chromatographic process was started after injecting a given eluent in a volume of 1 mL through an injection pipe into a mobile-phase application bar. Finally, the TLC plate was removed from the chamber module immediately after the mobile-phase front reached the plate edge located opposite to the application bar.

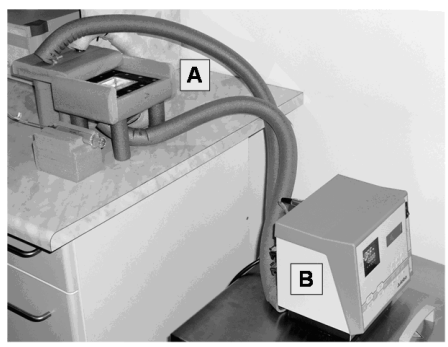

Fig. 1. Perspective view of the planar micro-chromatography device: horizontal chamber unit working inside temperature controlled metal oven (**A**), external liquid circulating thermostat (**B**).
Rys. 1. Widok ogólny zestawu do mikrochromatografii planarnej: horyzontalny moduł komory chromatograficznej umieszczony w bloku termostatycznym (**A**), zewnętrzny termostat cyrkulacyjny (**B**).

Chromatographic separations were performed under unsaturated chamber conditions using 3:7 (v/v) acetone:*n*-hexane and 8:2 (v/v) methanol:water mobile phases for spirulina extracts and sea trout bile samples, respectively. Spots patterns were acquired by direct scanning under visible light conditions and after application (by dipping method) of a visualization reagent consisted of 10% phosphomolybdic acid (PMA) in methanol. Under such conditions blue-gray colored spots were generated after the plates were dipped in the PMA reagent and heated at 60°C for 25 min. Additional spots, corresponding to visible light transparent substances, on the plates with spirulina samples were detected by iodine vapor exposure (room temperature; 30 min).

Picture acquisition was performed using a Plustek OpticPro S12 USB scanner (Plustek, Taipei, Taiwan) with an 8-bit per RGB channel color deep mode, 600 DPI resolution, and saved as TIFF files without compression with the help of image-acquisition software: Image Folio v. 4.2.0 (1991-2000, NewSoft Technology Corporation).

Fluorescence visualization for λ_{EX}=254 and 366 nm was performed using a Cobrabid UV lamp (Warszawa, Poland). For that purpose the TLC plate was placed on the black background 10 cm from the light source (the angle between lamp/plate/digital camera lens was 45° approximately). The chromatographic pattern observed under visible light was acquired using an Olympus Camedia 5050 Zoom, 5.0 Mega pixel digital camera (Olympus Optical Co. Ltd., Japan) equipped with a 43-mm UV filter (Marumi, Japan). The camera lens was positioned 9 cm above the TLC plate centre and digital shots were taken by using the following

camera settings: focusing mode manual (Super Macro Zoom), shutter speed 1/6s or 2s for λ_{EX}=254 and 366 nm, respectively, aperture F8.0, ISO sensitivity 64, recording mode RAW, image resolution 2,560×1,920. All Olympus RAW files (16 bits per RGB channel color deep mode) were transformed into an 8-bit TIFF file using Adobe Photoshop Software (San Jose, CA, USA).

After data acquisition an appropriate TLC plate area was cropped from the original frame size, and subsequently auto-balance, gray scale conversion and/or noise reduction filters were applied. Selected cross-sections of the chromatographic lanes were extracted from the digital pictures with the help of Scion Image freeware (Scion Corporation; ver. 4.0.3.2; http://www.scioncorp.com/).

2.3. Raw samples preparation and plates application protocols

Raw bile was collected from the sea trout (*Salmo trutta* m. *trutta*) (Fig. 2A). A liquid content of fish gallbladder (Fig. 2B) was directly transferred to the glass tube and sealed with PTFE lid. Collected bile samples were stored at temperature of -20°C, until sample proceeding. Unfrozen bile samples (100 μL) were lyophilized (Fig. 3) and deproteinized by solving of dry bile in methanol (ultrasonic bath, 15 min). In the resulting solution a dry bile content was 1 mg per 1 mL of methanol, approximately. Solid particles, which were present in the samples after methanol addition and sonication, were separated from the liquid by centrifugation (5800 rpm, 15 min). Clear bile solution was transferred onto the TLC plate start line. Freeze-drying procedure was performed using Refrigerated Vapor Traps RVT4104 (Thermo, Milford, MA, USA). Deep freeze (below -100°C) bile samples in glass containers were dried under vacuum at room temperature using vacuum centrifugal evaporator Savant SPD121P Speed Vac connected to VLP80 oil vacuum pump, which were products of Thermo, Milford, MA, USA.

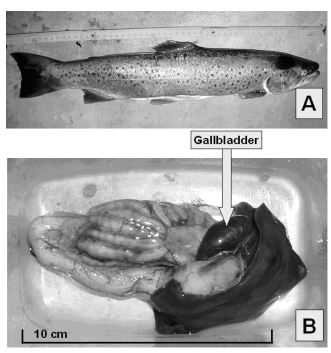

Fig. 2. Sea trout (*Salmo trutta* m. *trutta*) (**A**) and isolated part of digestive system including liver and gallbladder (**B**).
Rys. 2. Troć wędrowna (*Salmo trutta* m. *trutta*) (**A**) oraz wypreparowany fragment przewodu pokarmowego wraz z wątrobą i uwidocznionym pęcherzykiem żółciowym (**B**).

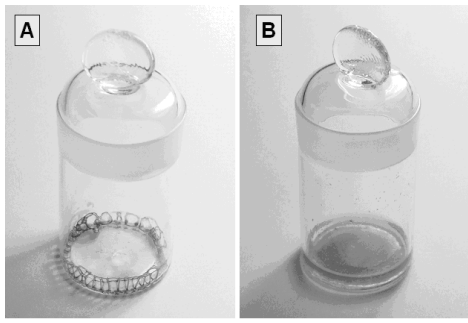

Fig. 3. Lyophilized (**A**) and re-solved in methanol (**B**) fish bile sample (raw bile volume = 100 μL)
Rys. 3. Liofilizowana (**A**) i rozpuszczona w metanolu (**B**) próbka żółci rybiej (objętość próbki żółci = 100 μL).

Cyanobacteria material (Fig. 4) from the Spirulina Pacifica pharmaceutical formulation (500 mg tablets consisting of *Spirulina platensis*, Cyanotech Corp. Kailua-Kona, Hawaii, USA) was powdered manually using a small ceramic mortar, and 150 mg samples were transferred into 5 mL glass tubes. The samples were mixed with 1 mL of the extraction liquid, which was methanol. Afterward, the tubes were sealed and sonicated for 1 h at room temperature using an ultrasonic bath. Next, the tubes were centrifuged and clear algae extract was transferred onto the TLC plate start line.

Fig. 4. „Spirulina" pharmaceutical medicines (**A**) mainly composed of dry cyanobacteria cells (**B**) incorporated into tablets and capsules (**C**)
Rys. 4. Preparaty farmaceutyczne „Spirulina" (**A**) zawierające wysuszone komórki cyjanobakterii (**B**) uformowane jako tabletki lub kapsułki (**C**)

Solid-phase extraction of target compounds ranging with polarity from estetrol to progesterone extracted from surface water, treated and untreated sewage waters samples were purified and concentrated using Supelclean LC-18 solid phase extraction (SPE) tubes (6 mL, 0.5 g) and 12-ports vacuum manifold obtained from Supelco (Bellefonte, PA, USA). Extraction procedure was performed according to an analytical protocol reported in our previous study concerning determination of endocrine disrupting compounds using temperature-dependent inclusion chromatography [12].

Micro-TLC plates were spotted with given samples using Linomat 5 semi-automatic application instrument (Camag, Switzerland), controlled through the Planar Chromatography Manager (winCATS software, 1999-2008, version 1.4.4.6337). Using the spray-on technique narrow 4 or 5 mm long bands were formed along start line that was located 5 mm from the bottom edge of TLC plate.

3. Results and discussion

In analytical practice, a modern high-performance thin-layer chromatography (HPTLC) involving reversed phase (RP) plate is particularly suitable for efficient separation and sensitive visualization of active compounds from complex mixtures [1, 2]. It is noteworthy that using HPTLC plates, the mobile phase developing distance can be reduced to less than 50 mm, which is well documented in the literature [3, 7, 13, 14]. This conclusion is based on the observation that minimum values of the plate height (H) can be achieved if the solvent migration distance along the HPTLC plate ranges from 30 to 40 mm [3]. Under such conditions total analysis time can be dramatically reduced in comparison to chromatographic separations performed on typical 10 or 20 cm long TLC plates. Our previous experimental data have revealed that two-dimensional (2D) developing protocol involving micro-HPTLC plate is capable to separate of more than 240 spots consisting of low molecular mass compounds like steroids or colored substances from cyanobacterial (*Spirulina maxima*) organisms [4-7].

Spirulina is a group of cyanobacteria that has attracted worldwide attention due to their utilization as human and animal nutritional protein sources and is the only cyanobacteria to be grown on an industrial scale. Spirulina possess a wide range of colored substances, including carotenoids, chlorophyll, and

phycobiliproteins, that can be used as food and cosmetic colorants. As can be seen from chromatograms and densitograms presented in Fig. 5, micro-TLC technique working in 1D mode is capable to separate whole range of colored substances from dry cells of *Spirulina platensis*, which were isolated by simple one-step extraction with methanol. Detection of target substances for *e.g.* fingerprinting or screening purposes as well as chemo-taxonomic studies can be significantly improved by exposure of developed micro-plates on iodine vapors at room temperature (Fig. 5B).

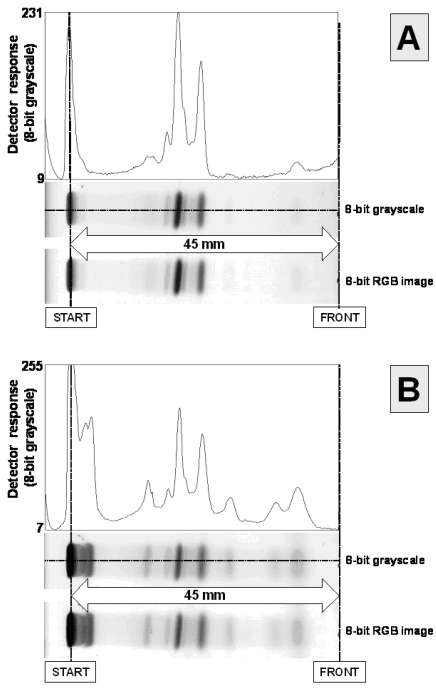

Fig. 5. Micro-TLC chromatogram of methanolic extract the *Spirulina platensis* dyes obtained from the Spirulina tablet. Analytical conditions: sample volume - 3μL; sample application - 5 mm band using spray-on technique; separation temperature +30°C; stationary phase - HPTLC RP18W; mobile phase composition - 30% (v/v) acetone/*n*-hexane; detection - visible light conditions (**A**) and after iodine vapor exposure in room temperature (**B**); chromatogram acquisition method - digital scan using Plustek OpticPro S12 USB office scanner. Densitograms corresponding to original chromatograms were derived using Scion Image

Rys. 5. Mikro-TLC chromatogram metanolowego ekstraktu barwników *Spirulina platensis* uzyskanego z tabletki preparatu farmaceutycznego Spirulina. Warunki wykonania analizy: objętość naniesionej próbki - 3μL; sposób naniesienia próbki - pasmo długości 5 mm techniką natryskową; temperatura procesu rozwijania +30°C; faza stacjonarna - HPTLC RP18W; skład fazy ruchomej - 30% (v/v) aceton/*n*-heksan; detekcja - bezpośrednia w świetle widzialnym (**A**) oraz po ekspozycji na pary jodu w temperaturze pokojowej (**B**); urządzenie rejestrujące chromatogram: obraz na płytce zeskanowano przy użyciu skanera biurowego Plustek OpticPro S12 USB. Densytogramy odpowiadające poszczególnym chromatogramom uzyskano za pomocą programu Scion Image

One of the advantages of planar chromatography over its column counterpart is that each TLC run can be performed using non-previously-used stationary phase. Therefore, it is possible to fractionate or separate complex samples characterized by heavy biological matrix loading. In case of column chromatography such samples must be carefully pre-purified and usually diluted. Particularly, all substances that are strongly adsorbed by stationary phase must be carefully removed to provide column long life. According to chromatogram presented in Fig. 6, wide range of complex sample quantity (fish bile) can be efficiently fractionated through micro-TLC process. In such case sample pre-treatment protocol, especially deproteinization step, was based on simple freeze-drying procedure (Fig. 3). As it is presented in Fig. 7, number of UV-Vis transparent substances including bile acids can be detected *via* fluorescence or staining with phosphomolybdic acid (PMA) procedures. Such method can be applied for sex-specific studies or intra- and inter-species comparisons.

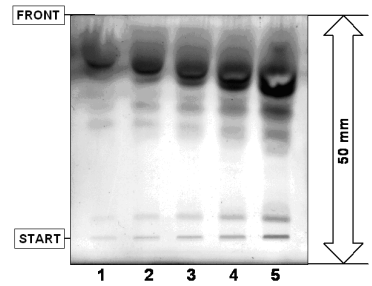

Fig. 6. Separation of main components of sea trout bile using different samples quantity: 1, 2, 3, 5 and 10 μL (lane No 1, 2, 3, 4 and 5, respectively) of deproteinized methanolic solution presented in Fig. 3. Analytical conditions: sample application - 5 mm band using spray-on technique; separation temperature +50°C; stationary phase - HPTLC RP18WF$_{254}$S; mobile phase composition - 80% (v/v) methanol/water; spots visualization: developed chromatogram dipped in 10% (w/v) phosphomolybdic acid (PMA) in methanol and heated for 25 min at 60°C; chromatogram acquisition method - direct digital scan under visible light conditions using Plustek OpticPro S12 USB office scanner

Rys. 6. Rozdzielenie głównych składników żółci troci wędrownej przy zastosowaniu różnych ilości próbki: 1, 2, 3, 5 oraz 10 μL (ścieżka nr 1, 2, 3, 4 oraz 5) odbiałczonego metanolowego roztworu przedstawionego na rys. 3. Warunki wykonania chromatogramu: sposób naniesienia próbki - pasmo długości 5 mm techniką natryskową; temperatura procesu rozwijania +50°C; faza stacjonarna - HPTLC RP18WF$_{254}$S; skład fazy ruchomej - 80% (v/v) metanol/woda; detekcja plamek: płytka z rozwiniętym chromatogramem zanurzona w 10% (w/v) metanolowym roztworze kwasu fosforomolibdenowego (PMA) i wygrzewana przez 25 min. w temperaturze 60°C; rejestracja chromatogramu: obraz na płytce zeskanowano przy użyciu skanera biurowego Plustek OpticPro S12 USB

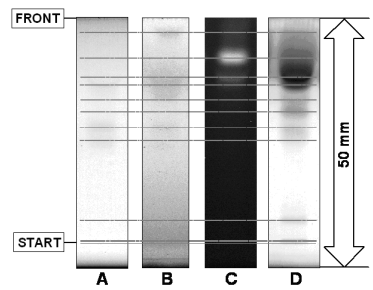

Fig. 7. Detection of main components of sea trout bile sample (3 μL) under different UV-Vis light exposure and visualization reagent condition: lane **A**: visible light, lane **B**: fluorescence (λ_{EX} = 254 nm; λ_{EM} = visible light), lane **C**: fluorescence (λ_{EX} = 366 nm; λ_{EM} = visible light), lane **D** visible light after phosphomolybdic acid staining; chromatogram acquisition method for fluorescence detection: 5.0 Mega pixel digital camera Olympus Camedia 5050 Zoom equipped with a 43 mm UV filter; remaining analytical conditions are similar to those specified within Fig. 6 caption

Rys. 7. Detekcja głównych składników próbki (3 μL) żółci troci wędrownej w warunkach różnego oświetlenia w zakresie UV-Vis oraz uwidocznienia plamek odczynnikiem wywołującym: ścieżka **A**: światło widzialne, ścieżka **B**: fluorescencja (λ_{EX} = 254 nm; λ_{EM} = światło widzialne), ścieżka **C**: fluorescencja (λ_{EX} = 366 nm; λ_{EM} = światło widzialne), ścieżka **D**: światło widzialne po wybarwieniu kwasem fosforomolibdenowym; rejestracja chromatogramu dla detekcji fluorescencyjnej: aparat cyfrowy 5 Mpix Olympus Camedia 5050 Zoom, wyposażony w filtr UV 43 mm; pozostałe warunki wykonania analizy analogiczne do wyszczególnionych w opisie rys. 6

Micro-chromatography can be also an effective tool for simple and rapid classification of the environmental samples characterized by different sources of endocrine disrupting compounds (EDCs). Similarly, to HPLC UV-Vis chromatographic profiles of solid-phase extracts obtained from environmental samples [12], micro-TLC separation involving PMA staining and fluorescence detection is capable for efficient fingerprinting and characterization of environmental samples. Particularly, individual band patterns for samples derived from surface water including Baltic Sea, selected lakes and rivers of the Middle Pomerania in northern part of Poland, as well as high organic load untreated and treated sewage water, can be easily observed on micro-TLC chromatograms (Fig. 8).

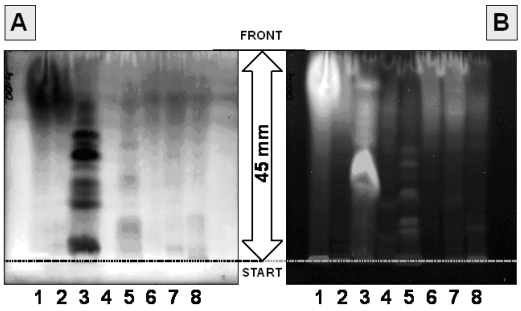

Fig. 8. Screening of environmental samples SPE extracts using visualization reagent - PMA (**A**) and fluorescence (λ_{EX} = 366 nm; λ_{EM} = visible light) detection (**B**). Samples I.D.: **1** - Lubiatowo Lake (SPE protocol without cleaning solvent step); **2** - Lubiatowo Lake (full SPE procedure); **3** - untreated sewage water; **4** - treated sewage water (municipal wastewater treatment plant „Jamno"); **5** - Baltic Sea; **6** - Kamienne Lake; **7** - Dzierżęcinka River (under Koszalin); **8** - Jamno Lake. Analytical conditions: samples volume - 5 µL, sample application - 4 mm band using spray-on technique, separation temperature +30°C, stationary phase - HPTLC RP18WF$_{254}$S, mobile phase composition - 80% (v/v) methanol/water; chromatogram acquisition method - direct digital scan under visible light conditions using Plustek OpticPro S12 USB office scanner (PMA plate); fluorescence detection: 5.0 Mega pixel digital camera Olympus Camedia 5050 Zoom equipped with a 43 mm UV filter

Rys. 8. Porównanie ekstraktów SPE próbek środowiskowych przy użyciu odczynnika wybarwiającego – PMA (**A**) oraz fluorescencji (λ_{EX} = 366 nm; λ_{EM} = światło widzialne) (**B**). Numeracja próbek: **1** - Jezioro Lubiatowo (procedura SPE bez cieczy czyszczącej); **2** - Jezioro Lubiatowo (pełna procedura SPE); **3** - ściek surowy; **4** - ściek oczyszczony (Oczyszczalnia Ścieków Komunalnych „Jamno"); **5** - Morze Bałtyckie; **6** - Jezioro Kamienne; **7** - rzeka Dzierżęcinka (za Koszalinem); **8** - Jezioro Jamno. Warunki wykonania chromatogramu: objętość naniesionych próbek - 5 µL, sposób naniesienia próbki - pasmo długości 4 mm techniką natryskową, temperatura procesu rozwijania +30°C, faza stacjonarna - HPTLC RP18WF$_{254}$S, skład fazy ruchomej - 80% (v/v) metanol/woda; rejestracja chromatogramu: obraz na płytce po zastosowaniu PMA zeskanowano skanerem biurowym Plustek OpticPro S12 USB; detekcja fluorescencyjna: aparat cyfrowy 5 Mpix Olympus Camedia 5050 Zoom, wyposażony w filtr UV 43 mm

4. Conclusions

The main advantages of non-forced flow rate planar chromatography result from its simplicity, easy operation and the inexpensive equipment needed. The results of our experiments indicate that in particular cases fractionation and/or separation as well as characterization of complex raw biological materials and the environmental samples *via* analytical protocol involving temperature-controlled planar micro-chromatography (micro-TLC) may be simple and non-expensive alternative for fingerprinting protocols based on HPLC machines equipped with UV-Vis detectors. Particularly, it has been demonstrated that using micro-TLC plates with fluorescence indicator a sensitive detection of target compounds for fast screening of complex materials like fish bile or environmental SPE extracts can be successfully performed.

5. References

[1] Sherma J., Fried B.: Handbook of Thin-Layer Chromatography, 3rd ed., Marcel Dekker, New York, 2003.
[2] Waksmundzka-Hajnos M. (Editor), Sherma J., Kowalska T.: Thin Layer Chromatography in Phytochemistry, CRC Press, Taylor and Francis Group, 2008.
[3] Wall P.E.: Thin-Layer Chromatography. A Modern Practical Approach, Springer/Royal Society of Chemistry, Cambridge, 2006.
[4] Zarzycki P.K., Zarzycka M.B., J. AOAC Int.: Association of Analytical Communities International, 91, 2008.
[5] Zarzycki P.K., Zarzycka M.B., Głód B.K.: Miesięcznik PAK, Wydawnictwo Pomiary Automatyka Kontrola, 55, 2009.
[6] Zarzycki P.K., Zarzycka M.B.: Anal. Bioanal. Chem., Springer, 391, 2008.
[7] Zarzycki P.K.: J. Chromatogr. A, Elsevier, 1187, 2008.
[8] Martinez A.W., Phillips S.T., Whitesides G.M., Carrilho E.: Anal. Chem., ACS Publications, 82, 2010.
[9] Morlock G., Schwack W.: Anal. Bioanal. Chem., Springer, 385, 2006.
[10] Brzezinka H., Bertram N.: J. Chromatogr. Sci., Preston Publications, 40, 2002.
[11] Crecelius A., Clench M.R., Richards D.S.: Curr. Trends Mass Spectrom., Advanstar Communications, 19, 2004.
[12] Zarzycki P.K., Włodarczyk E., Baran M.J.: J. Chromatogr. A., Elsevier, 1216, 2009.
[13] Reich E., Blatter A., Meier B.: TLC for the Analysis of Herbal Drugs, Camag Scientific Note, Muttenz, 2003, available at: http:// www.camag. ch/index.php.
[14] Chomicki A., Ślązak P., Dzido T.H.: Electrophoresis, Wiley InterScience, 30, 2009.

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