

Research Paper

Separation and determination of the group-type composition of modern base and lubricating oils with a wide range of polarity, especially emitted to the environment

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Abstract

Lubricating oils are composed of base oils (> 85% v/v) and enriching additives (<15% v/v). Three types of base oils may be distinguished: 1) traditional bases (obtained by low-volatile fractions from crude oil distillation refining), 2) synthetic bases (mainly poly-alpha-olefins, sometimes esters, especially succinic acid esters), 3) bases of natural origin (especially obtained from refined plant oils). The bases of natural origin are the only ones recommended for application when lubricating oil may be emitted to the environment (e.g. when the machine with an open cutting system is used). Group-type separation and analysis of group-type composition of base and lubricating oils are of significant importance in quality control and environmental monitoring. Due to the potentially wide range of polarity of the components of base and lubricating oils, group-type separation becomes a difficult separation problem. It is also a serious analytical problem due to the considerable diversity of physicochemical properties. The authors propose a new procedure for the separation and determination of the group-type composition of base and lubricating oils using thin-layer liquid chromatography in normal phase systems (abr. NP-TLC) on silica gel plates impregnated with berberine salt/ in the coupling of thin-layer chromatography with flame ionization detection (abr. TLC-FID). A new, effective procedure of TLC plates impregnation with berberine sulphate was presented. The proposed procedure ensures the visualization of all groups of base oils. Extensive experimental research showed that a 2-step development procedure with application of n-hexane up to 100% height of development + 15 min and further n-hexane: isopropanol: tri-fluoroacetic acid 96.25: 3: 0.75 (v: v: v) up to 75% height of development is advantageous for the group-type separation, both in TLC-FID and TLC.

Keywords: lubricating oil; base oil; group – type composition; TLC; TLC-FID; berberine impregnation

1. Introduction

Lubricating oil is considered as a fluid structural element of machines (consisting of base oil 85% v/v and a package of enriching additives up to 15% v:v) [1,2]. There are three main groups of base oils: traditional base oils (so-called mineral), synthetic base oils and natural base oils [2]. Mineral base oils [1,3] contain low-volatile, liquid, branched aliphatic hydrocarbons, mainly isoalkanes (Paraffins, abr. *P*), aliphatic substituted alicyclic hydrocarbons, the so-called naphthenes (abr. *N*), aliphatic and/ or alicyclic substituted aromatic hydrocarbons, mainly single-ring aromatics (abr. *A*), as well as very low levels of polar organic chemicals, resins (abr. *R*) [4]. The lowest polar fraction of saturated hydrocarbons is often called saturates (abs. *S*). Saturates are the sum of paraffins and naphthenes, $S = P + N$. The enriching packages fulfil various utility functions, such as: anti-seizing, anti-corrosive, removing deposits, anti-foaming, accelerating deemulsification with water, improving heat exchange and others. They are always products of the organic synthesis industry.

Mineral base oils produced from crude oil, as well as lubricating oils produced on its basis, are harmful both to the environment and to the health of the device operator or people in the range of the mist sprayed by the machine [5]. The oil mist arises during the use of cutting devices, as well as

during pouring oils into transport containers [6]. A worker exposed to oil mist has a high risk of skin cancer, pathological changes in the lungs, liver, kidneys and heart, as well as irritable and allergic reactions [7, 8]. When mineral oils are emitted to the environment, they undergo multi-stage changes as a result of interaction with soil components, water and sunlight. As a consequence, changes in the chemical structure occur, leading to formulation of secondary transformation products that may increase the level of eco-toxicity of the emitted substance [9, 10].

Synthetic base oils represent the second group of base oils. The main and most frequently used representatives of this group are the poly-alpha-olefins (*PAO*), less frequently synthetic esters, especially succinic acid esters or polyesters [1,11,12]. Due to their relatively high price, synthetic base oils are currently used as motor oils and as gear oils. The third group of base oils are the base oils of natural origin, especially refined plant oils and their derivatives. These oils should be used exclusively in lubricating oils emitted to the environment or potentially capable of polluting the environment [13]. Natural origin oils consist mainly of triacylglycerols (abr. *TAG*), diacylglycerols (abr. *DAG*) and monoacylglycerols (abr. *MAG*). Some manufacturers also declare to add free fatty acid esters, especially fatty acid ethyl esters (abr. *FAE*), sometimes methyl esters (abr. *FAME*) to the lubricating oils composed of natural oil bases. As a result of hydrolysis, natural oil bases may contain free fatty acids (abr. *FFA*), polyunsaturated free fatty acids (abr. *PUFA*) and glycerol (abr. *GL*). Waxes of plant origin, mainly squalane and squalene [14] may also occur. Oils of natural origin have favourable performance properties [2], they are however susceptible to hydrolysis and have a low oxidative resistance [15]. They are also more expensive than mineral oils obtained from petroleum. What is especially important, they are easily biodegradable [14,16,17]. When hydraulic oil may leak onto the soil during a breakdown of the device, it should also be considered to apply natural base oil, which would be harmless to the environment [5,16,17,18].

Group- type separation and analysis of group-type composition of base and lubricating oils is essential in the quality control of lubricating oils, as well as in environmental monitoring. The literature on the subject is dominated by group-type separation and determination methods based on adsorption - desorption in glass columns with a polar porous adsorbent, or with several such adsorbents, and gravimetric determination of the weight fraction of individual fractions. [19-21].

Conditions that allow the very low polar saturated hydrocarbons (*S*) to be separated from the slightly more polar low volatile aromatic hydrocarbons (*A*) in the base oils include thermally activated polar adsorbents such as silica gel, alumina, so-called *Attapuscus clay* (natural adsorbent) and the use of a very low-polar solvent (n-pentane (*n-C5*) or n-heptane (*n-C7*)), sometimes n-hexane (*n-C6*) for *S* and *A* separation [22–26]. Group-type separation of the *S/ A* components is very difficult using both adsorption-desorption and HPLC due to the steric obstacles occurring in the molecular structure of components [22,23,26]. Neither of these methods [27-32] enable the separation and determination of the group-type composition of base oils containing a matrix including acylglycerols and their derivatives [32-35]. The development must be either stepwise or gradient to specify: *S/ A/ FAE/ TAG/ FFA/ DAG/ MAG* and *GL* groups.

In this study, the possibility of using Normal Phase - Thin Layer Chromatography (*NP-TLC*) and Thin Layer Chromatography coupled with Flame Ionization Detection was carried (*TLC-FID*).

Thin Layer Chromatography (abr. *TLC*) is a separation technique that has been used for several years [36,37]. The coupling of thin-layer liquid chromatography with flame-ionization detection (abr. *TLC-FID*) is a separation analytical technique for quantitative separation and determination of the detailed or group-type composition. Separation and determination of non-volatile or low-volatile mixtures, volatile organic chemical compounds, including those of natural origin, mixtures with a relatively wide range of polarity via *TLC-FID* is broadly described in the literature [38-42]. The literature describes many methodologies of using *TLC-FID* to study the composition of lubricating oils [43], heavy crude oil distillates and tar [44], petroleum origin products [45], heavy oils and tars [46]. In cases where *TLC* or *TLC-FID* are used to separate and determine the group-type composition of mixtures with a relatively wide range of polarity and a relatively complicated composition, i.e. for separation and determination of the group-type composition of asphalts or residues from vacuum distillation of crude oil, lipids and phospholipids, the most commonly used is stepwise two- or three-stage development [47]. The solvent with the lowest solvent strength has the longest development path, and the solvent with the highest solvent strength - the shortest [48,49]. The issues related to an explanation of the correct sequence of the stepwise development for group-type

separation specifying *S/ A/ FAE/ TAG/ FFA/ DAG / MAG/ GL* groups when some components do not dissolve in one of the solvents of the stepwise development program are an object of this paper.

The subject of this study is the development of new methodologies for group-type separation and determination of group-type composition of base and lubricating oils with a wide range of polarity of components with application of i./ stepwise development with the *NP-TLC* technique on silica gel plates impregnated with a salt of berberine to assess the group-type composition of the oil, as well as in the pilot studies for *TLC-FID*; ii./ *TLC-FID* separation and analytical techniques to determine the group-type composition of oils. The research also includes the problem of the correct sequence of the stepwise development in the case of the lack of solubility of glycerol and monoacylglycerols and their derivatives in volatile aliphatic hydrocarbons constituting one of the solvents in the stepwise development program.

2. Materials and methods

2.1 Reagents and samples

Silica GEL 60 F254s 200x200mm TLC plates supported on aluminium foil were applied Merck, Darmstadt, Germany. Berberine sulphate for impregnation (purity $\geq 98\%$, was purchased in Sigma-Aldrich, Tokyo, Japan,); methanol, (purity for LC) was purchased in Merck Darmstadt, Germany. Following TLC and TLC-FID solvents components were applied: n-hexane (n-C6) (purity for HPLC, Merck Darmstadt, Germany), isopropanol (IsoOH. purity for HPLC, Merck Darmstadt, Germany), tert-butyl methyl ether (abr. MTBE; purity for HPLC, Merck Darmstadt, Germany), chloroform (analytical purity, POCH, Gliwice, Poland), dichloromethane (abr. DCM; analytical purity, Chempur, Piekary Śląskie, Poland), toluene (purity for HPLC, Merck Darmstadt, Germany), methanol (purity for HPLC, Merck Darmstadt, Germany), trifluoroacetic acid (abr. TFA, purity for analysis; Merck Darmstadt, Germany).

Following sample solvents: dichloromethane (abr. DCM; analytical purity; Chempur, Piekary Śląskie, Poland); acetone (analytical purity; Chempur, Piekary Śląskie, Poland) were used.

Crystalline iodine for visualisation (I_2) was purchased from Sigma-Aldrich, Poznań, Poland (analytical purity).

The samples are summarized in Table 1. The concentration of the samples was set at a given level according to the principle: sample material weight / sorbent weight $< 10^{-4}$ g/ g.

Table 1. Sample summary.

No.	Characteristics of the sample	Acronym	Purity, Producer (if applicable), City, Country	Sample concentration
1.	Anhydrous glycerol	GL	Analytical purity, POCH, Gliwice, Poland	5 mg/mL acetone
2.	Monoacylglycerol 1-monopalmoyleoyl-rac-glycerol	MAG	Purity $\geq 99\%$, Sigma-Aldrich, Darmstadt, Germany	7 mg/mL acetone
3.	Diacylglycerol 1,2-dioleoyl-rac-glycerol	DAG	Purity $\geq 99\%$, Sigma-Aldrich, Darmstadt, Germany	5 mg/mL acetone
4.	Edible rapeseed oil	TAG1	100% plant oil, Szamotuły, Poland	5 mg/mL DCM
5.	Edible sunflower oil	TAG2	100% plant oil, Bolesław, Poland	5 mg/mL DCM
6.	TAG1+TAG2 1:1 (v:v)	TAG	Mixture of samples 4 and 5	5 mg/mL DCM
7.	Free Fatty Acid, Oleic Acid	FFA	Purity $\geq 99\%$, Sigma-Aldrich, Darmstadt, Germany	5 mg/mL DCM
8.	Fatty Acids Methyl Esters	FAME	Technical purity, LOTOS SA, Gdańsk, Poland	5 mg/mL DCM
9.	Machine oil produced on the basis of crude oil processing	LAN	Commercial product, Orlen, Płock, Poland	5 mg/mL DCM
10.	SAE 30/90 base oil from petroleum refining; SAE30 consists of S and A in proportions S:A equal to 3:1 by mass	S30	Technical purity, LOTOS, Gdańsk, Poland	5 mg/mL DCM
11.	PAO 6 synthetic oil base of	PAO	Technical purity, Chevron Phillips Chemical, Texas, USA	5 mg/mL DCM

	polyalphaolefin type, 100% S			
12.	Squalene polyunsaturated hydrocarbon, triterpene	Sq	Purity $\geq 99\%$, Sigma-Aldrich, Darmstadt, Germany	5 mg/mL DCM
13.	Plant-based lubricating oil with a package of conditioners, commercially available	O1	Commercial product, Gliwice, Poland	5 mg/mL DCM
14.	Fry; Edible plant oil – exhausted sample	O2	Exhausted oil based on 100% plant oil, Szamotuły, Poland	5 mg/mL DCM
15.	High-quality chain saw oil, commercially available	O3	Commercial product, Gliwice, Poland	5 mg/mL DCM
16.	Motor oil, synthetic polyalphaolefin type, commercially available	O4	Commercial product, Warszawa, Poland	5 mg/mL DCM
17.	Fully biodegradable Preservative lubricant for saw cutting systems, new formulation	O5	Commercial product, Malbork, Poland	5 mg/mL DCM
18.	Lubricating oil for chainsaws, commercially available	O6	Commercial product, Malbork, Poland	5 mg/mL DCM
19.	Hydraulic oil	H	Technical purity, LOTOS, Gdańsk, Poland	5 mg/mL DCM
20.	Turbine Oil	T	Technical purity, LOTOS, Gdańsk, Poland	5 mg/mL DCM
21.	SAE 10/90 Base oil from petroleum refining	S10	Technical purity, LOTOS, Gdańsk, Poland	5 mg/mL DCM
22.	Brighthstock Base oil derived from the refining of vacuum crude oil residues	BS	Technical purity, a LOTOS, Gdańsk, Poland	5 mg/mL DCM

DCM – dichloromethane; following substances were used as technical standards: S10 (for S and A, since S30 consists of S and A in proportions S:A equal to 3:1 by mass), PAO (for S); mixture of TAG1 and TAG2 (1:1 v/v) for TAG.

• *TLC separation*

TLC separation was carried in Glass Developing Chamber with dimensions 250x50x200mm (Duran, Lingea Chemicals, Warsaw, Poland) with a glass cover; three walls were lined with cellulose filter paper (Merck Darmstadt, Germany).

Sampling was carried with a 5 μ L glass micro-syringe in the volume of 3 μ L (Hamilton, Nevada, USA), the minimal dimensions of the spot were kept under a TLC dryer: TK-8 (Iatron Labs, Tokyo, Japan). The concentration of the samples is given in Table 1. Samples were placed- at a distance of 1.5 cm from the edges of the plate and with the distance 1 cm between the spots.

The dried TLC plates with the applied samples were suspended for 10 minutes above the developing solvent height (9 mm) in a closed TLC chamber. This ensured an equilibrium state between the solvent vapours in the TLC developing chamber and on the sorption surface. The plate development heights are given in Tables 2, 3 and 4 depending on the development method used. After each development step on a specific solvent, the TLC plate was dried (105°C for 15 min, until constant weight). The visualization was carried under UV lamp (TB 02, UV TB Telebid, Warsaw, Poland) and subsequently the TLC plate was placed in a desiccator over crystalline iodine (30 g I₂/ L) for 15 min, in temperature 35 \pm 2°C. The activities described above were performed under a fume laboratory extractor. Pictures of the TLC plates after each visualisation step were taken with XIOAMI Camera (Redmi A2 Lite, 12MPix, Hon Hai Precision Industry Co., Shenzhen, China).

Table 2. Development conditions for TLC and TLC-FID chromatograms in 1-step TLC development (to 100% of the development height)

One-component solvents	n-C6; cyclohexane; (MTBE), chloroform, DCM
Two-component solvents	n-C6:IsoOH [v:v]: 99.7:0.3; 99.4:0.6; 98.75:1.25; 97.5:2.5; 95:5; 90:10; 70:30
Three-component solvents	nC6:IsoOH:MTBE [v:v:v] 98.7:0.3:1.0; 98.4:0.6:1.0; 97.8:1.25:1.0; 96.5:2.5:1.0; 87.5:5:7.5; 40:10:50

MTBE – methyl-tertbutyl-ether; DCM- dichloromethane; IsoOH- isopropanol; n-C6 – n-hexane

As shown in tables 3 and 4, the stepwise development was performed, either by increasing solvent strength of the solvent in subsequent development stages, while reducing the development distance along the height of the adsorbent layer on the TLC plate, or by decreasing the solvent strength of the solvent in subsequent development stages, while increasing the distance of development along the height of the TLC plate.

Table 3. Development conditions for TLC and TLC-FID chromatograms in 2-step development.

Acronym	2-stage development method
A1	1. n-C6 up to 100% of development height
	2. n-C6: IsoOH: TFA 94.25:5:0.75 (v:v:v) up to 70% of development height
B1	1. n-C6: IsoOH: TFA 94.25:5:0.75 (v:v:v) up to 70% of development height
	2. n-C6 up to 100% of development height + 10 min (TLC-FID)/ + 5 min (TLC)
A2	1. n-C6 up to 100% of development height +10 min
	2. n-C6: IsoOH: TFA 96.25:3:0.75 (v:v:v) up to 70% of development height
B2	1. n-C6: IsoOH: TFA 96.25:3:0.75 (v:v:v) up to 70% of development height
	2. n-C6 up to 100% of development height +10 min
A3	1. n-C6 up to 100% of development height +15 min (TLC-FID)/ + 7.5 min(TLC)
	2. n-C6: IsoOH: TFA 96.25:3:0.75 (v:v:v) up to 75% of development height
B3	1. n-C6: IsoOH: TFA 96.25:3:0.75 (v:v:v) up to 60% of development height
	2. n-C6 up to 100% of development height + 5 min (TLC-FID)/ + 2.5 min (TLC)

n-C6 – n-hexane; IsoOH- isopropanol; TFA- trifluoroacetic acid

In tables 3 and 4 the conditions defined by acronym A and C refer to the first development step using the solvent with the lowest solvent strength. This step was developed up to 100% of the height of the TLC plate sorption layer. The last step was carried with the solvent with the highest solvent strength while the shortest migration distance along the height of the TLC plate sorption layer was applied. The conditions defined by acronym B and D refer to the first development step using the solvent with the highest solvent strength, while the shortest migration distance along the height of the TLC plate sorption layer was applied. The last step was carried with the solvent with the lowest solvent strength within 100% of the height of the TLC plate.

Table 4. Development conditions for TLC and TLC-FID chromatograms in 3-step development.

Acronym	3-stage development method
C1	1. n-C6 up to 100% of development height +10 min (TLC-FID)/ + 5 min (TLC)
	2. toluene up to 60% of development height
	3. DCM:MeOH 95:5 (v:v) up to 40% of development height
D1	1. DCM:MeOH 95:5 (v:v) up to 40% of development height
	2. toluene up to 70% of development height
	3. n-C6 up to 100% of development height + 10 min (TLC-FID)/ + 5 min (TLC)
C2	1. n-C6 up to 100% of development height +10 min
	2. toluene up to 60% of development height
	3. CHCl ₃ :MeOH 97:3 (v:v) up to 50% of development height
D2	1. CHCl ₃ :MeOH 97:3 (v:v) up to 50% of development height
	2. toluene up to 60% of development height
	3. n-C6 up to 100% of development height +10 min

n-C6 – n-hexane; MeOH- methanol; DCM - dichloromethane

For impregnation purposes, TLC plates were placed under a fume laboratory extractor on the table surface on cellulose blotting paper and impregnated with a 60 mg/ mL solution of berberine sulphate in methanol by spraying it with a glass TLC sprayer placed approx. 30 cm above the plate (Erlenmeyer flask with a capacity of 100 mL, with NS 19/26 socket, Duran spray head (Lenz Laborglasinstrumente, Darmstad, Germany). A volume of 50 mL of the methanol berberine sulphate solution was used for each plate. The plates were dried in a laboratory dryer (model 30l Pro, Adverti , Łódź, Poland) at 105°C until a constant mass was reached (1 hour). The plate was then transferred to a desiccator with



desiccant until the TLC plate cooled down. The plates were weighed on an analytical scale (Radwag, Model: SBS-LW-300A, Warsaw, Poland) with an accuracy of 0.1 mg.

After each development step, the plate surface was photographed under a UV lamp in the light of $\lambda = 254$ nm and $\lambda = 365$ nm, the borders of the spots were pencil-marked. Further, the plate was exposed to iodine vapour for 10 minutes in a closed desiccator at room temperature. After exposition, the plates were photographed.

On the basis of the obtained chromatograms, the values of R_f and retention factors (k) were determined for individual spots - representing certain substances or groups of chemical compounds.

The R_f factor was calculated using equation 1:

$$R_f = a/b \quad (\text{eq. 1})$$

where:

a - distance from the starting point to the point of the highest intensity of colour / fluorescence spots on the TLC chromatogram, mm

b - distance passed by the moving phase until the end of the development zone, mm

In the case of development above the height of the development zone, the distance was defined as the length of the adsorbent layer on the TLC plate/ TLC FID rod. This approach is not common and usually prohibited, however due to the circumstances described in p.3.4 is accurate and allows to obtain better S/A resolution during two-step development.

Retention factor was determined on the basis of R_f and can be calculated using equation 2:

$$k = (1-R_f) / R_f \quad (\text{eq. 2})$$

where:

k - retention factor, -

The selectivity factor was determined on the basis of the ratio of the retention factors obtained under the same conditions, according to equation 3 [50]:

$$\alpha = k_2/k_1 \quad (\text{eq. 3})$$

where:

α - selectivity factor, -

k_1 - retention factor for a faster spot (migrating higher) on the TLC plate / TLC-FID rod, -

k_2 - retention factor for a slower spot (migrating lower) on the TLC plate / TLC-FID rod, -

To compare the obtained values with the use of non-impregnated and impregnated TLC plates, the range (abr. R) was determined as the difference between the values of the retention factors for individual groups of chemical compounds according to equation 4:

$$R = | (k_j - k_i) | \quad (\text{eq. 4})$$

where:

k_j - value of the retention factor for a specific chemical compound/ group of chemical compounds (for specific spot on the TLC chromatogram) with a non-impregnated adsorbent, -

k_i - value of the retention factor for a specific chemical compound/ group of chemical compounds (for specific spot on the TLC chromatogram) with the impregnated adsorbent, -

Values of the solvent strength of one-component solvents and multi-component solvents components relative to silica gel (ϵ^0) were adopted from literature [33].

TLC-FID separation

For TLC-FID separation, the following set was applied: a ten-rod frame with 10 TLC-FID - chromarod-S III® (micron-sized silica gel with a special preparation and a very high specific surface: above 1000 m²/ g); TLC-FID analyser with flame-ionizing detector: (Iatroscan, Iatron Labs, Tokyo, Japan); measuring analogue-digital transmitter: A/ C-4 (with a measuring range: +/- 5μV to +/- 5V, sampling frequency 40 Hz, - AKORD, Toruń, Poland); Data processing software from the A/C-4 transducer: "Chomik-2" version for Windows -(developed originally in Gdańsk University of Technology, Gdańsk, Poland); sample dispenser SES 3200 / IS-01 (Iatron Labs, Tokyo, Japan); glass developing chamber with dimensions 140x200x50mm with three walls lined with cellulose tissue, with a glass cover, a handle for a frame with Chromarod-S III rods and the adjustment system for the

frame position along the chamber height; (Duran, Lingea Chemicals, Warsaw, Poland); frame with TLC-FID rods / TLC plates dryer: TK-8, (Iatron Labs, Tokyo, Japan).

The Chromarod-S III frame was activated by firing the rods once at 35 seconds/ 10 cm and twice at 50 seconds/ 10 cm on a TLC-FID analyser (i.e. carrying each rod by hydrogen air flame before dispensing the solution of the mixture separated)). Activated frame was placed in a desiccator with an active drying agent for 10 minutes until temperature reached the ambient temperature. Further the samples were loaded onto the TLC-FID frame rods with the automatic sample dispenser. 0.5 μl of the sample was placed at the concentration given in Table 1. After the samples were applied, the TLC-FID frame was placed in a laboratory drier at 105°C for 10 minutes. After complete evaporation of the solvent, the TLC-FID frame plate was placed in a desiccator with an active drying agent.

To establish an equilibrium state between the solvent vapours in the TLC chamber and on the sorption surface, the dried frame with TLC-FID rods loaded with samples was suspended for 10 minutes above the solvent level (20 mm above the bottom of the chamber). The frame with the TLC-FID rods was next immersed in the solvent with the bottom edge of each sample spot above the solvent level. Further, the solvent reached the height given in Tables 2, 3 and 4 depending on the development method used. After each development step, the frame with TLC-FID rods was dried (105°C for 15 min, until constant weight). The activities described above were performed under a fume laboratory extractor. After complete evaporation of the solvent, the TLC frame was placed in a desiccator with an active drying agent.

After the last development step the frame with the rods was placed in an Iatroscan analyser with a flame ionization detector (TLC-FID analyser) and chromatograms were recorded. The flow rate of hydrogen was equal to 150 mL/ min; the flow rate of air was 1.8 L/ min. The displacement of the rods in the flame of the TLC-FID detector: 50 s/ 10 cm. The peak areas were digitally separated.

Based on the maximum of each peak from the TLC-FID highlighted in the individual chromatograms (fig. 4), the R_f values, the retention factor (k) and the selectivity factors (α) were calculated (please, see eq. 1-4). The names of individual chemicals/ groups of chemicals were assigned based on the retention parameters obtained under TLC conditions on plates impregnated with berberine sulfate, under the same development conditions. The limits of detection (LOD) were determined based on the interpolation of the regression function for individual groups of chemical compounds. The noise amplitude was determined and was equal to 0.05 μV (mean value obtained from 6 noise heights in the chromatogram). The average noise amplitude was multiplied 3 times (0.15 μV). Then, on the basis of the calibration curves (x - mass of the substance, μg ; y - peak height for a given substance, μV), the LOD was determined as the height of the peak that exceeds the noise value for a given substance. The limits of quantification (LOQ) were determined as equal to three times LOD. In order to evaluate the dispersion of the values of the retention factor (k) and standard deviation were calculated. The ranges of SD values determined for $k \in (1.5; 5)$ are given in tables 5 and 6 for the results performed in triplicates. The calibration curve method (External Standard) was applied, assuming a linear course of the calibration function forcing 0.0 point.

3. Results and Discussion

3.1 *TLC plates impregnation methodology*

The only known method of obtaining visualization of aliphatic or alicyclic molecular structures in the VIS range on *TLC* plates with silica gel is the use of impregnation of the plate [50], which may be carried with berberine salt. Molecular structures enhancing the fluorescence of berberine are present in all groups of chemical compounds of base and lubricating oils with a wide range of polarity, except glycerol. The studies (Table S.1) have shown that in the case of glycerol or other low volatile alcohols or polyols, the berberine salt exhibits a dark blue to navy blue fluorescence. This determines the possibility of identifying the presence with the use of *TLC* plates impregnated with berberine salt, as well as an approximate assessment of the group-type composition of base and lubricating oils with a wide range of polarity of components.

The berberine salt was evenly distributed on the plate surface. The differences in the mass of the plates after impregnation were in the range 0.1-0.8 mg, i.e. no more than 1% of the total weight of berberine sulphate applied on the *TLC* plate (Table S.1). Exemplary *TLC* plates impregnated with berberine

sulphate are presented in figure 1. The range of retention factors (k) for components migrated with the solvent with low solvent strength did not exceed 0.05, and for the solvent with higher solvent strength it did not exceed 0.03. It can be concluded that the impregnation of *TLC* plates with silica gel F-254s with berberine sulphate, performed according to the method developed in this study, has no effect on retention for solvents with low and medium-low solvent strength used in this study. It can also be stated that the developed impregnation methodology allows to obtain an even distribution of berberine sulphate over the entire surface of the *TLC* plates. As shown in Figure 1 (C and D plates), despite the high content of saturated hydrocarbons in oils, the visualization of *S* group hydrocarbons after exposure to iodine vapours is practically non-existent. It is also very weak for group *A* with the above described molecular structures sterically hindering the addition of iodine to the aromatic ring. At the same time, it is highly sensitive to acylglycerols and derivatives containing unsaturated fatty acids. The study of this work also showed that impregnation of the *TLC* plate with berberine sulphate increases the intensity of the colour of the spots caused by its adducts with iodine of mono-, di- or tri-substituted acylglycerols or esters containing unsaturated fatty acids. On the other hand, it is almost impossible to visualize saturated fatty acids, their esters with aliphatic alcohols, or acyl-glycerols in I_2 vapours. These chemicals are visible on *TLC* plates impregnated with berberine sulphate if they contain aliphatic or alicyclic ($-CH_2-CH_2-$) structures in the molecules. Our studies have shown that in the case of glycerol or other low volatile alcohols or polyols, the berberine salt exhibits a dark blue to navy blue fluorescence. This determines the possibility of identifying the presence as well as an indicative assessment of the group-type composition of base and lubricating oils with a wide range of component polarity specifying the *S/ A/ TAG/ FAE/ FFA/ DAG/ MAG/ GL* groups, as confirmed by the research presented in this work.

It should also be added that the intensity of the changes in berberine fluorescence intensity caused by aliphatic molecular structures is not high. Consequently, visualization is only possible for relatively high mass of components/ groups of components within the *TLC* spots. For groups of hydrocarbons migrating with a low polar alkane, i.e. for *S*, the *LOD* value can be estimated at 0.5 mg, for aromatic hydrocarbons *A* at 0.5 mg. For groups migrating with a higher polar solvent, the *LOD* values are higher, i.e. for *FAME* - *LOD* is around 0.7 mg, for *TAG*: approx. 1 mg, for *FFA*: 1 mg, *DAG*: 1 mg, *MAG*: 1 mg. On the other hand, for glycerol *GL* remaining at the dosing point, due to the intense navy blue fluorescence of berberine, *LOD* is approximately equal to 1 mg [51].

3.2 Research on retention and selectivity of *TLC* separation with the use of single/ multi-component solvents and single and multi-stage development

The groups of substances that may constitute or represent components of the base or lubricating oils used in cutting machines are listed in Table S.2. The results are shown in Table S.2 as the values of the retention factors k calculated from R_f for silica gel F254s impregnated with berberine sulphate.

Certain groups of organic chemicals were eluted/ separated as specific chemical compounds that could constitute components or represent appropriate groups of components/ components of base or lubricating oils, especially oils for cutting machines. Squalene can also be treated as a low volatile alkene (so-called olefin) while *GL* is a product of complete hydrolysis of acyl-glycerols and represents low-volatile polar polyols and alcohols solvent strength

Single-component solvents

Based on the results presented in Table S.2 and previous studies [21, 35, 47, 52,53], it can be assumed that the solvent with a low solvent strength (i.e. n-hexane, *n-C6*; $\epsilon^o = 0.00$ [34]) is the only single-component solvent enabling the separation of the saturated hydrocarbons group ($S = P + N$) from aliphatic and alicyclic substituted aromatic hydrocarbons *A* from base/ lubricating oils ($\alpha_{A/S} = 2.70$). An alkane based solvent will therefore be necessary in one of the group -type separation steps of the base/ lubricating oils. However, the solvent strength of n-C6 or any other alkane is so low that it does not even allow the migration of acyl-glycerols and their derivatives from the starting point. Moreover, n-hexane is a bad solvent for *DAG* and *FFA*, and it does not completely dissolve *MAG* and *GL* [54]. One-component solvents characterized by a higher solvent strength than alkanes, which would enable the migration and separation of acyl-glycerols and their derivatives, i.e. *MTBE* ($\epsilon^o = 0.20$ [34]), or dichloromethane ($\epsilon^o = 0.33$ [34]) - do not allow separation of saturated hydrocarbons *S* from



aromatics *A* ($\alpha_{A/S} = 1.00$). Hence the conclusion that the group-type separation procedure by *TLC / TLC -FID* technique of base and lubricating oils with a wide range of polarity must include a development step using n-C6 can be drawn. Most likely, iso-alkanes such as iso-octane will also be suitable. In addition, it was found that application of cyclo-alkanes such as cyclohexane or methylcyclopentane also allows *S/ A* separation in base and lubricating oils under *TLC/ TLC-FID* conditions, but with lower selectivity than using alkanes as solvent. The separation procedure cannot therefore be a one-step process. Therefore, at least a two-step separation procedure is necessary. Moreover, the step with the alkane as solvent to separate *S* and *A* should be carried to obtain the longest possible development distance. The selectivity of *S/A* group-type separation of the components of base and lubricating oils is advantageous under the conditions of *TLC* or *TLC - FID*.

Two-component solvents

In the second stage of the research, two-component solvents with n-C6 as one of the solvent components were included. Isopropanol (iso-OH) in various proportions was added to n-hexane (as the component with the highest volume fraction). Isopropanol is a component with relatively high solvent strength ($\varepsilon^o = 0.63$ [34]), and at the same time a relatively good solvent for organic chemical compounds from low-polar to polar. By increasing the proportion of the more polar component in the solvent, the elution force of the mobile phase is increased and the nature of the adsorption interactions on the sorption surface changes. The conditions of this stage of the research are listed in Table 3, and the results are presented in Table S.2.

On the basis of these results it can be concluded that the *MAG* migration from the starting point is possible only when the volumetric *iso-OH* content in the solvent is higher than 5% (v/ v). Below this content, $k_{MAG} \Rightarrow \infty$. At the same time, the maximum isopropanol content for the spots of the saturated and aromatic hydrocarbon groups which did not overlap on the *TLC* chromatograms is 0.75% (v/ v). In addition, isopropanol is a solvent component that adversely affects the separation of *FAME* (low molecular weight esters of alcohols and fatty acids) from triacylglycerols (*TAG*). The spots were practically overlapping.

Careful analysis of the data in the second part of Table S.2 leads to the conclusion that the two-component n-hexane: isopropanol solvent in any proportion of these components is not selective enough to enable separation on *TLC* plates (hence also in the *TLC-FID*) under one-stage development conditions ensuring the possibility of determination of the group-type composition with respect to *S/ A/ Sq/ FAE / TAG/ FFA/ DAG/ MAG/ GL groups*.

Three-component solvents

The results of the studies on the use of the three-component solvents are presented in the third part of Table S.2. n-hexane is used as the solvent component with the highest volume fraction and isopropanol as the component with relatively high solvent strength. *MTBE* was applied as the medium solvent strength additive. The possibility of using an isocratic mixture of non-polar n-hexane with slightly more polar *MTBE* and very little addition of relatively polar isopropanol in the development step of separating acyl-glycerols from their derivatives was investigated.

Under *TLC* stepwise development conditions, chloroform as a medium-polar addition to the solvent should ensure group -type separation of base and lubricating oils [24,55]. Even though, chloroform was not used as a component of the solvent for several reasons, i.e.: high toxicity and ecotoxicity and especially low durability of $CHCl_3$ in the air atmosphere - with the formation of phosgene without the addition of the so-called polar stabilizer.

As can be seen in Table S.2, the isopropanol - *MTBE* mixture in a favourable proportion can replace chloroform in the group-type separation of base and lubricating oils containing acylglycerols and their derivatives. The solvent, which enables *S/ A* group-type separation of oils via *TLC* on silica gel plates, is a mixture of n-C6: *Iso-OH: MTBE* in the ratio 98.7: 0.3: 1 (v: v: v) ($\alpha_{A/S} = 1.71$) However, a preferred solvent that allows the separation of acylglycerols and their derivatives with respect to the *Sq/ FAE/ TAG/ DAG/ FFA/ MAG/ GL groups* ($\alpha_{MAG / DAG} = 5.39$; $\alpha_{DAG/FFA} = 1.67$; $\alpha_{FFA/FAME} = 1.85$; $\alpha_{TAG/FAME} = 1.04$; $\alpha_{FAME/A} = 2.35$) is n-C6: *Iso-OH: MTBE* in the ratio 87.5: 5: 7.5 (v: v: v)

The test results also show that for the separation of base and lubricating oils with respect to the *S/ A/ Sq/ FAE/ TAG/ FFA/ DAG/ MAG/ GL groups*, at least two-stage development is necessary. In the development step, which should ensure *S/ A* group-type separation, n-hexane or another alkane

should be used as a one-component solvent and the development distance should be as long as possible. For the separation of acylglycerols and their derivatives, the above-mentioned multi-component solvent is advantageous. At the same time, the distance of the development with a solvent with a higher solvent strength than the alkane should be as high as possible. It should be so determined that the group of aromatic hydrocarbons *A* forms one spot without degrading the *S/ A* resolution. Thus, in this step, a separation of *A/ Sq/ FAE/ TAG/ FFA/ DAG/ MAG/ GL* (*GL* spot remaining at the starting point) with sufficient resolution should occur.

3.3 Research on the development of favourable conditions for group-type separation of base/lubricating oils using the TLC technique

In the case of group-type separation of components of materials with a relatively complex group-type composition using the *TLC* or *TLC-FID* technique, containing aliphatic hydrocarbons *S* and aliphatic / alicyclic substituted single and multi-ring aromatic hydrocarbons *A*, the literature most often proposes a three-stage separation with decreasing the development distance and increasing the solvent strength of the solvent in the subsequent development stages [22,39,48]. Possibly longest migration distance is preferred for *S/ A* group-type separation with an alkane (here *n-C6*) as solvent. For the migration and separation of acyl-glycerols and their derivatives, an solvent with higher solvent strength than the alkanes (i.e. *n-C6*) is obviously needed. To ensure migration of relatively polar *MAGs* and polar glycerol (*GL*), the solvent strength of the solvent should be high.

The research includes several different procedures for a two-stage and three-stage development for group-type separation of base and lubricating oils with a wide range of polarity of components. Various solvents were tested using an solvent with higher solvent strength than the alkanes. The different distance of *TLC* expansion in the different development steps were taken into account. The test conditions are presented in Tables 3, 4, and 7. Separation of the *S/ A/ Sq/ FAE/ TAG/ FFA/ DAG/ MAG/ GL* groups was investigated with multi-component solvents composed of: *n-C6*, *MTBE*, *iso-OH*. Additionally, a very little addition of trifluoroacetic acid (*TFA*) was investigated. *TFA* plays the role of blocking "overactive" sites on the adsorption surface, which positively affects the spot width of acylglycerols and derivatives, especially *FFA* [56] (when *n-C6* was used as solvent). The apparent displacement of the *n-C6* front above the upper limit of the adsorbent layer on the *TLC* plate (also - *TLC-FID* rod) was taken into account. This provides an extension of the migration path (time) of the *S* and *A* groups and improved group-type separation.

The results of these studies are shown in Table S.3. To draw attention to the reverse migration order of specific components or groups of components, the selectivity factor α (calculated as defined from the ratio of the retention factors of the later eluted to the earlier eluted spot) is distinguished in Table S.3 with an asterisk symbol (*). Figures 1, 2 and 3 are examples of the developed *TLC* plates taking into account different conditions for the visualization of spots on *TLC* plates.

The separation results given in Table S.3 were used to design the preferred group-type separation conditions by the *TLC-FID* technique. They were also taken into account in formulating other various conclusions from the research of this work, including the issue of stepwise sequence in the study of group-type composition of oils with a wide range of polarity of components.

Sequence of stepwise development

Development of the *TLC / TLC-FID* chromatograms was performed in two paths:

- increasing the solvent strength of successive solvents and at the same time decreasing the development distance in subsequent stages of the stepwise development (the first stage with an alkane as the solvent at the maximum development distance, the next - with an solvent with increased solvent strength at a shorter distance with inter-stages plate drying to remove traces of the solvent between successive development stages);
- decreasing the solvent strength of the solvent and increasing the migration distance in subsequent stages.

Detailed results are presented in Table S.3. A comparison of the chromatograms obtained using the same 2-stage development conditions; with different sequences of changes in solvent strength is presented in Figure 2. It is observed that the order of the change of solvent strength in successive development step has a significant effect on the migration and group-type separation of low-polar *S/ A* groups under *TLC* conditions. This may be related to the different activity of the adsorbent surface

during the development step with alkane solvent. When the original *TLC* plate (or the frame with *TLC-FID* rods) is exposed in a *TLC* chamber to an atmosphere of nonpolar alkane vapours and the first development step is performed with this solvent, greater retention and higher selectivity of *S* and *A* separation occurs (i.e. *TLC* plates A, C in the Fig. 2.). In other case, when the exposure of the *TLC* plate (or the frame with *TLC-FID* rods) takes place in the vapours of the more polar solvent components, it leads to a certain degree of deactivation of the silica gel adsorption surface and, at the same time, it lowers the values of the retention factors *k* of low-polar components of the groups *A* and *S* (including B and D *TLC* plates in the Fig. 2).

3.4 the two and three step *TLC* development efficiency

Based on the results in Table S.2 and the exemplary illustration in Fig. 1. It can be observed that the three-step development procedure proposed in the literature for the batch separation of asphalts or heavy petroleum products containing asphaltene [20,47] is not effective for the group-type separation of base and lubricating oils by a wide range of polarity. This occurs irrespective of whether the successive stepwise development steps are carried out in the direction of increasing or decreasing the solvent strength of the solvent. There is no *TLC* plate with sufficient separation selectivity when the individual development steps are performed with alkane up to 100% of the adsorbent layer distance, toluene up to 60 - 70% of the distance and: *DCM* / *MeOH* in the ratio 95: 5 v / v up to about 30 - 40% of the distance of the adsorbent layer and irrespective of whether there is a decrease or an increase in the solvent strength of the solvent in the subsequent development steps.

The studies and research described above and the analysis of their results lead to a general conclusion that the two-stage development procedure should be the most appropriate for the group-type separation of base and lubricating oils with respect to *S*/ *A*/ *Sq*/ *FAE*/ *TAG*/ *FFA* *DAG*/ *MAG*/ *GL* groups (*GL* remains at the dosing point). At the same time, the greatest possible migration path should be ensured in each of the development and separation steps.

Studies have shown that extension of the development time with an alkane over the development zone of the *TLC* plate/ *TLC-FID* rods for a certain time is advantageous to improve *S* and *A* resolution during the extension; the low-polar volatile solvent is vaporized from the top edge of the adsorbent on the *TLC* plate/ *TLC-FID* rod. The development time extension value varies for *TLC* and *TLC-FID* as well as for increasing or decreasing the solvent strength of the solvent in the subsequent development steps. Appropriate values should be selected experimentally, ensuring that the top of the *S* group spot does not reach the upper limit of the development zone on the *TLC* plate/ *TLC-FID* rod.

3.5 Research on the selection of favourable separation conditions in *TLC-FID* with the use of pilot tests using the *TLC* technique

As stated in the introduction, it is very important to be able to determine the group-type composition of base/ lubricating oils with a wide range of polarity, especially oils emitted to the environment from various types of cutting machines, as well as oils that can potentially become a significant pollution of the environment as a result of machine failure. The studies with the use of *TLC* showed that the *TLC-FID* technique could be appropriate for this purpose. To develop a favourable separation and analytical procedure the results of the *TLC* tests were used. The results given in Table S.3 for which representative *TLC* developed plates are shown in Figure 3 are particularly interesting for this purpose. The chromatograms obtained using the *TLC-FID* technique for several different group-type separation conditions determined on the basis of the *TLC* test results are presented in Figure 4. Table S.3 presents the results of the *TLC-FID* tests as the values of the retention factors *k* calculated on the basis of *R_f* from the *TLC-FID* chromatograms. Application of the same development conditions in *TLC* (Figure 3) and *TLC-FID* (Figure 4) allowed to obtain the same development sequence of the separated components. The values of the retention and selectivity parameters are similar, but not identical (Table S.3). Therefore, it is possible to minimize the research on the selection of favourable separation conditions, performed directly by the *TLC-FID* technique. It should also be added that the development time of the n-hexane (n-C6) front to 100% of the distance of the *TLC* plate with a length of 10 cm development zone is approx. 13 min and for *TLC-FID* rods with a length of 10 cm of the adsorbent layer, approx. 26 min. Therefore, the additional n-C6 development time for *TLC* was reduced twice as compared to *TLC-FID* (Table S.3). *TLC-FID* chromatograms presented in Figure 4 are performed under different development conditions given in Table 4. It can be seen that the

preferred conditions for separation and group-type composition determination of base/ lubricating oils with a wide range of polarity of components are the two-stage development conditions coded as A3 and B3. Specifically:

- A3 - first development step: n-C6 to 100% development height + 15min for *TLC-FID* (+ 7.5min for *TLC*); second development step: n-C6: *IsoOH*: *TFA* 96.25: 3: 0.75 (v: v: v) up to 75% of development height;

- B3 - first development step: n-C6: *IsoOH*: *TFA* 96.25: 3: 0.75 (v: v: v) up to 60% of the development height; second step: n-C6 to 100% development height + 5min for *TLC-FID* (+ 2.5min for *TLC*).

Due to the obtained results, the conditions under A3 procedure seem slightly more favourable than B3. In practice, they can be used alternatively, especially for the low sample concentrations during the first separation step using an alkane as solvent, no adverse effects were observed due to the insolubility of certain polar components of the oil in the low-polar solvent.

Based on the results of the research presented in Figure 4, it can also be concluded that the problem of low solubility of certain oil components in the low-polar solvent during the first separation step seems to be of secondary importance for oils that are liquids at room temperature and since small masses of samples are applied to *TLC-FID* rods (*TLC* plates). Under conditions of larger sample masses (higher degree of adsorbent overload), the B3 method will probably be more advantageous than A3.

3.6 Validation parameters of the *TLC-FID* methodology and results of determination of group-type composition

The validation parameters for determination of the mass of components/ groups of components of base/ lubricating/ edible oils using the A3 methodology in the *TLC-FID* technique are presented in Table 5.

Table 5. Summary of statistical parameters for determination of the mass of a component/ group of components in group –type separation by *TLC-FID* technique by A3 method under the conditions described in Table 4.

Group		GL	MAG	DAG	FFA	FAME	TAG	Sq	A	S
Regression equation	-	$y=1.61x$	$y=1.74x$	$y=1.13x$	$y=1.33x$	$y=1.09x$	$y=1.38x$	$y=1.94x$	$y=0.52x$	$y=2.72x$
R ²	-	0.97	0.99	0.98	0.99	0.98	0.99	0.98	0.99	0.99
LOQ	µg	0.09	0.09	0.13	0.11	0.14	0.11	0.08	0.03	0.06
LOD	µg	0.27	0.27	0.39	0.33	0.42	0.33	0.24	0.09	0.18

y - height of the peak of a specific component / group of components in the *TLC-FID* chromatogram [µV]; x-mass of a specific component/ group of components in the sample dosed onto the *TLC-FID* rod [µg].

The equations of the calibration functions were determined on the basis of the dependence of height of the respective peaks as a function of the mass of a component/ group of components in the *TLC-FID* chromatograms. The methodology for the calibration curve determination (External Standard) was applied, assuming a linear course of the calibration function forcing (0,0) point. Such approach is generally accepted and correct in the case of *TLC-FID* [40].

The A3 methodology (Table 4, Figures 3 and 4) was used to investigate the group-type composition of selected commercially available base or natural lubricating oils. The results of these studies are summarized in Table S.4. The data in Table S.4 were obtained by the *TLC-FID* technique and the A3 method, based on the integration of all peak areas on the *TLC-FID* chromatograms. The names were assigned to the specific peaks based on the order of migration of the respective components/ component groups and the values of the respective retention parameters on *TLC*.

The results in Table S.4 are adequate for petroleum, synthetic and semi-synthetic base oils, as well as for natural origin oils and their conversion products. For lubricating oils containing additives, there are additional peaks from these "additives", but also an overlap of peaks from the additives on the peaks from base oil components may occur. The results presented in Table S.4 are indicative for the description of the group-type composition of the oil.

To determine the content of petroleum fractions in the tested oil, containing a group of saturated hydrocarbons that are very difficult to biodegrade *S* and a group of aromatic hydrocarbons *A* that are extremely harmful to the environment and health, the obtained results are of high importance. In order to identify the presence and an approximate assessment of the content of petroleum fractions in the lubricating oil, it is particularly important to test the oil by *TLC* technique with the use of plates impregnated with berberine salt. Only the identification of *S* group in oil, in the absence of the *A* group, proves that the base oil is a *PAO* type synthetic oil. The lack of *S* and *A* groups and a high content of the *TAG* group at the same time, prove that the base oil is purely plant oil of natural origin. Then, depending on the content of the *FFA* group, one can obtain information about the degree of the plant oil hydrolysis process.

4. Conclusions

Methodologies for the separation and assessment of the group-type composition of base and lubricating oils with a wide range of polarity of components were developed with respect to *S/ A/ Sq / FAE/ TAG/ FFA/ DAG/ MAG/ GL groups* (*GL* spot remains at the starting point), using thin-layer liquid chromatography (*TLC*) on silica gel impregnated with berberine salt thin-layer chromatography coupled with flame ionization detection (*TLC - FID*). As a result of the research, favourable group-type separation conditions were determined.

In the step using a completely non-polar solvent (n-C6) with the longest possible development path, a separation of low volatile, mainly branched aliphatic hydrocarbons *P* and aliphatic substituted alicyclic *N* oil hydrocarbons occurred. Extension of the time and hence the development path, in the non-polar solvent application stage is of significant importance for obtaining possibly selective *S* and *A* group-type separation. The extension value is experimentally selected, while observing the upper limit of the *S* group spot almost reaches the upper limit of the adsorbent layer on the *TLC* plate and also on a *TLC-FID* rod. (5 min for *TLC* and 15 min for *TLC-FID* under applied experiment conditions). The migration when medium-polar solvent is applied for the development and separation of acylglycerols and their derivatives should be as long as possible, but without deteriorating the degree of *S* and *A* group-type separation.

During multistep development, the solvent primarily used may be an alkane, despite the fact that it has virtually no *MAG* solubility and very low *FFA*'s solubility. However, when adsorbent overload occurs, the solvent used in the first separation step should be a good solvent for all components of the separated mixture. Differences in adsorbent properties on *TLC* F-254 plates and Chromarod SIII rods make it impossible to directly transfer the values of retention and selectivity parameters from *TLC* to *TLC-FID*. The order of migration and the values of the retention and selectivity parameters are however similar in both techniques. The impregnation of the *TLC* plates with berberine sulphate is necessary for the visualization of all groups of oil components in *UV* 365nm light. For some groups, especially those containing alkenes or unsaturated fatty acids or their derivatives, the sensitivity of visualization with iodine is higher than that obtained for the same groups after impregnation with berberine sulphate. A fluorescence at 365 nm light of dark blue to navy blue occurs due to presence of adducts between glycerol and berberine. This provides the visualization of this group of chemicals on the plates impregnated with the berberine salt.

The obtained research results presented in this work and the conclusions are particularly important for the assessment of environmental and health harmfulness of lubricating oils emitted to the environment from various types of cutting machines, as well as hydraulic oils that may potentially become environmental pollution as a result of machine failure. Determination of the presence and of the content of petroleum components in the oil is possible. The procedure also enables the assessment of the degree of hydrolytic degradation of triacylglycerols. Hence the procedure may be applied for the assessment of the "degree of degradation" of oils of natural origin. The obtained procedures may be used in the technical analysis of group-type composition of all types of base and lubricating oils: from crude oil refining, refined plant oils and their derivatives, synthetic oils, as well as various mixtures of the above-mentioned oils.

Ethics declarations

No violation of human/ animal rights occurred during this investigation.

Ethics approval

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Conflict of interest

The authors declare no competing interests nor conflict of interest.

Supplementary Materials

The Authors provide Supplementary Materials (1 file).

References

1. Beran, E. Wpływ budowy chemicznej bazowych olejów smarowych na ich biodegradowalność i wybrane właściwości eksploatacyjne (*eng. Influence of the chemical structure of base lubricating oils on their biodegradability and selected operational properties*); Wyd. PWR: Wrocław, **2008**; ISBN 1896-4532.
2. Syahir, A.Z.; Zulkifli, N.W.M.; Masjuki, H.H.; Kalam, M.A.; Alabdulkarem, A.; Gulzar, M.; Khuong, L.S.; Harith, M.H. A review on bio-based lubricants and their applications. *J. Clean. Prod.* **2017**, *168*, 997–1016.
3. Haus, F.; Boissel, O.; Junter, G.A. Primary and ultimate biodegradabilities of mineral base oils and their relationships with oil viscosity. *Int. Biodeterior. Biodegrad.* **2004**, *54*, 189–192.
4. Weber, S.; Schmidt, T.; Schumacher, P.; Kuballa, T.; Mildau, G.; Walch, S.G.; Hartwig, A.; Lachenmeier, D.W. Quantification of Mineral Oil Aromatic Hydrocarbons (MOAH) in Anhydrous Cosmetics Using ¹H NMR. *J. Chem.* **2019**, *2019*, 1–10.
5. Nowak, P.; Kucharska, K.; Kamiński, M. Ecological and Health Effects of Lubricant Oils Emitted into the Environment. *Int. J. Environ. Res. Public Health* **2019**, *16*, 3002.
6. Gawęda, E.; Bednarek, K.; Szydło, Z. Oznaczanie mgły olejowej w powietrzu na stanowiskach pracy metodą wagową (*eng. Determination of oil mist in the air at workplaces using the gravimetric method*). *Bezpieczeństwo Pr.* **2005**, *12*, 11–14.
7. Aluyor, E.O.; Orijesu, M. Biodegradation of mineral oils – A review. *African J. Biotechnol.* **2009**, *8*, 915–920.
8. Krzemińska, S.; Irzmańska, E. Zagrożenia olejami mineralnymi na stanowiskach pracy oraz nowe rozwiązania polimerowych materiałów ochronnych w wybranych środkach ochrony indywidualnej (*eng. Threats with mineral oils at workplaces and new solutions of polymer protective materials in selected personal protective equipment*). *Med. Pr.* **2011**, *62*, 435–443.
9. Abosede, E.E. Effect of Crude Oil Pollution on some Soil Physical Properties. *IOSR J. Agric. Vet. Sci.* **2013**, *6*, 14–17.
10. Dmochowska, A.; Dmochowski, D.; Biegugnis, S. Charakterystyka biorekultywacji gleb skażonych produktami ropopochodnymi metodą pryzmowania *ex situ* (*eng. Characteristics of bio-cultivation of soils contaminated with petroleum products with application of ex situ prilling method*). *Annu. Set Environ. Prot.* **2016**, *18*, 759–771.
11. Wu, M.M.; Ho, S.C.; Forbus, T.R. Synthetic Lubricant Base Stock Processes and Products. In *Practical Advances in Petroleum Processing*; Springer New York: New York, NY, 2007; pp. 553–577.
12. Leslie R. R. Synthetics, Mineral Oils, and Bio-Based Lubricants: Chemistry and Technology; Chemical Industries, Ed.; 2nd ed.; CRC Press, 2013; ISBN 9781439855379.
13. Organization for Economic Co-operation and Development (2006) OECD Guidelines for the Testing of Chemical, Paris, France; 2006;
14. Buchgraber, M.; Ulberth, F.; Emons, H.; Anklam, E. Triacylglycerol profiling by using chromatographic techniques. *Eur. J. Lipid Sci. Technol.* **2004**, *106*, 621–648.
15. Jokai, R. Use of Vegetable-Based Hydraulic Oil in Forestry Operations: An Evaluation; Canada, 1995; Vol. SR-103

16. Adhvaryu, A.; Liu, Z.; Erhan, S.Z. Synthesis of novel alkoxyated triacylglycerols and their lubricant base oil properties. *Ind. Crops Prod.* **2005**, *21*, 113–119.
17. Cecilia, J.A.; Ballesteros Plata, D.; Alves Saboya, R.M.; Tavares de Luna, F.M.; Cavalcante, C.L.; Rodríguez-Castellón, E. An Overview of the Biolubricant Production Process: Challenges and Future Perspectives. *Processes* **2020**, *8*, 257.
18. Luna, F.M.T.; Cavalcante, J.B.; Silva, F.O.N.; Cavalcante, C.L. Studies on biodegradability of bio-based lubricants. *Tribol. Int.* **2015**, *92*, 301–306.
19. Bissada, K.K. (Adry); Tan, J.; Szymczyk, E.; Darnell, M.; Mei, M. Group-type characterization of crude oil and bitumen. Part I: Enhanced separation and quantification of saturates, aromatics, resins and asphaltenes (SARA). *Org. Geochem.* **2016**, *95*, 21–28.
20. Fan, T.; Buckley, J.S. Rapid and Accurate SARA Analysis of Medium Gravity Crude Oils. *Energy & Fuels* **2002**, *16*, 1571–1575.
21. ASTM D 2007:2019 Standard Test Method for Characteristic Groups in Rubber Extender and Processing Oils and Other Petroleum-Derived Oils by the Clay-Gel Absorption Chromatographic Method.
22. Gudebska J.; *Chromatografia cieczowa w oznaczaniu składu grupowego olejów bazowych i asfaltów drogowych (eng. Application of liquid chromatography in the determination of group-type composition of base oils and road bitumens, Doctoral Dissertation)*; Rozprawa Doktorska, 1999;
23. Kartanowicz, R. *Wysokosprawna chromatografia cieczowa w oznaczaniu składu grupowego oraz w analityce technicznej i procesowej produktów naftowych (eng. High performance liquid chromatography in the determination of group-type composition as well as in technical and process analysis of petroleum products, Doctoral Dissertation)*; Rozprawa Doktorska, Politechnika Gdańska: Gdańsk, 2004;
24. Stołyhwo, A. *Analityczne kryteria jakości zdrowotnej oleju z wiesiołka i innych bio-olejów z surowców naturalnych (eng. Analytical health quality criteria for evening primrose oil and other bio-oils from natural resources)*. *Camera Separatoria* **2016**, *8*, 112–118.
25. Snyder, L.R. *Principles of Adsorption Chromatography*; Marcel Dekker, Inc.: New York, 1968;
26. Kamiński, M.; Kartanowicz, R. Studies on selectivity of hydrocarbon group-type separation of petroleum vacuum distillates and base oils by HPLC. *Chem. Analityczna* **2003**, *48*, 531–542.
27. Ashoori, S.; Sharifi, M.; Masoumi, M.; Mohammad Salehi, M. The relationship between SARA fractions and crude oil stability. *Egypt. J. Pet.* **2017**, *26*, 209–213.
28. Silva, S.L.; Silva, A.M.S.; Ribeiro, J.C.; Martins, F.G.; Da Silva, F.A.; Silva, C.M. Chromatographic and spectroscopic analysis of heavy crude oil mixtures with emphasis in nuclear magnetic resonance spectroscopy: A review. *Anal. Chim. Acta* **2011**, *707*, 18–37.
29. Kamiński, M. *Studies on Selectivity of Hydrocarbon Group-Type Separation of Petroleum Vacuum Distillates and Base Oils by HPLC*. *Chem. Analityczna* **2003**, *48*, 531–542.
30. Pei, P.; Britton, J.; Hsu, S. Hydrocarbon Type Separation of Lubricating Base Oil in Multigram Quantity by Preparative HPLC. *J. Liq. Chromatogr.* **1983**, *6*, 627–645.
31. Robbins, W.K. Quantitative Measurement of Mass and Aromaticity Distributions for Heavy Distillates 1. Capabilities of the HPLC-2 System. *J. Chromatogr. Sci.* **1998**, *36*, 457–466.
32. Salimon, J.; Salih, N.; Yousif, E. Biolubricants: Raw materials, chemical modifications and environmental benefits. *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 519–530.
33. Nahar, A.; Baker, A.L.; Nichols, D.S.; Bowman, J.P.; Britz, M.L. Application of Thin-Layer Chromatography-Flame Ionization Detection (TLC-FID) to Total Lipid Quantitation in Mycolic-Acid Synthesizing *Rhodococcus* and *Williamsia* Species. *Int. J. Mol. Sci.* **2020**, *21*, 1670.
34. Meyer, V.R. *Practical High-Performance Liquid Chromatography*; 5th ed.; Wiley: Switzerland, 2010; ISBN 9780470682180.
35. Kamiński, M.; Kartanowicz, R.; Przyjazny, A. Application of high-performance liquid chromatography with ultraviolet diode array detection and refractive index detection to the determination of class composition and to the analysis of gasoline. *J. Chromatogr. A* **2004**, *1029*, 77–85.
36. *Thin-Layer Chromatography: A Modern Practical Approach* By Peter E. Wall. Royal Society of Chemistry: Cambridge. 2005. *Org. Process Res. Dev.* **2006**, *10*, 849–849.

37. Petruczynik, A.; Waksmundzka-Hajnos, M.; Hajnos, M.Ł. The Effect of Chromatographic Conditions on the Separation of Selected Alkaloids on Silica Layers. *J. Planar Chromatogr. – Mod. TLC* **2005**, *18*, 78–84.
38. Heep, J.; Tuchecker, P.H.K.; Gebhardt, C.R.; Dürr, M. Combination of Thin-Layer Chromatography and Mass Spectrometry Using Cluster-Induced Desorption/Ionization. *ACS Omega* **2019**, *4*, 22426–22430.
39. Vela, J.; Membrado, L.; Cebolla, V.L.; Ferrando, A.C. Suitability of thin-layer chromatography-flame ionization detection with regard to quantitative characterization of different fossil fuel products. II. Calibration methods concerning quantitative hydrocarbon-group type analysis. *J. Chromatogr. Sci.* 1998, *36*, 487–494.
40. Kim, E.; Cho, E.J.; Moon, S.; Park, J. II; Kim, S. Characterization of Petroleum Heavy Oil Fractions Prepared by Preparatory Liquid Chromatography with Thin-Layer Chromatography, High-Resolution Mass Spectrometry, and Gas Chromatography with an Atomic Emission Detector. *Energy and Fuels* 2016, *30*, 2932–2940.
41. Indrasena, W.M.; Henneberry, K.; Barrow, C.J.; Kralovec, J.A. Qualitative and Quantitative Analysis of Lipid Classes in Fish Oils by Thin-Layer Chromatography with an Iatroscan Flame Ionization Detector (TLC-FID) and Liquid Chromatography with an Evaporative Light Scattering Detector (LC-ELSD). *J. Liq. Chromatogr. Relat. Technol.* 2005, *28*, 2581–2595.
42. Banerjee, A.K.; Ratnayake, W.M.N.; Ackman, R.G. Effect of oxalic acid impregnation of chromarods on the separation of phospholipids for determination by the Iatroscan TLC/FID. *Lipids* 1985, *20*, 121–125.
43. Nowak, P.; Kosińska, J.; Glinka, M.; Kamiński, M. The Thin-Layer Microchromatography (μ TLC) and TLC–FID Technique as a New Methodology in the Study of Lubricating Oils. *J. AOAC Int.* **2017**, *100*, 922–934.
44. Barman, B.. Hydrocarbon-Type Analysis of Base Oils and Other Heavy Distillates by Thin-Layer Chromatography with Flame-Ionization Detection and by the Clay. *J. Chromatogr. Sci.* **1996**, *34*, 219–225.
45. Ali, M.; Hassan, A. Hydrocarbon group types analysis of petroleum products: a comparative evaluation of HPLC and TLC analytical performance. *Pet. Sci. Technol.* **2002**, *20*, 771–782.
46. Jiang, C.; Larter, S.R.; Noke, K.J.; Snowdon, L.R. TLC–FID (Iatroscan) analysis of heavy oil and tar sand samples. *Org. Geochem.* **2008**, *39*, 1210–1214.
47. Kamiński, M.; Gudebska, J.; Górecki, T.; Kartanowicz, R. Optimized conditions for hydrocarbon group type analysis of base oils by thin-layer chromatography–flame ionisation detection. *J. Chromatogr. A* **2003**, *991*, 255–266.
48. IP 469/01 (2006) Determination of saturated, aromatic and polar compounds in petroleum products by thin layer chromatography and flame ionization detection. **2006**.
49. Cossío, F.P.; Arrieta, A.; Cebolla, V.L.; Membrado, L.; Domingo, M.P.; Henrion, P.; Vela, J. Enhancement of Fluorescence in Thin-Layer Chromatography Induced by the Interaction between *n*-Alkanes and an Organic Cation. *Anal. Chem.* 2000, *72*, 1759–1766.
50. Skorupa, A.; Gierak, A. Detection and visualization methods used in thin-layer chromatography. *J. Planar Chromatogr. – Mod. TLC* 2011, *24*, 274–280.
51. Nowak, P.; Kucharska, K.; Kaminski, M.A. The New Test Procedure for Group-Type Composition of Base Oils of Lubricating Oils, Especially Emitted into the Environment. *Energies* 2020, *13*, 3772.
52. PKN PN EN 12916; 2016;
53. Vela, J.; Cebolla, V.L.; Membrado, L.; Andres, J.M. Quantitative hydrocarbon group type analysis of petroleum hydroconversion products using an improved TLC-FID system. *J. Chromatogr. Sci.* 1995, *33*, 417–425.
54. Johnson, L.A.; Lusas, E.W. Comparison of alternative solvents for oils extraction. *J. Am. Oil Chem. Soc.* 1983, *60*, 229–242.
55. Myher, J.J.; Kuksis, A. General strategies in chromatographic analysis of lipids. *J. Chromatogr. B Biomed. Sci. Appl.* 1995, *671*, 3–33.
56. Li Vigni, M.; Baschieri, C.; Marchetti, A.; Cocchi, M. RP-HPLC and chemometrics for wheat flour protein characterisation in an industrial bread-making process monitoring context. *Food Chem.* 2013, *139*, 553–562.

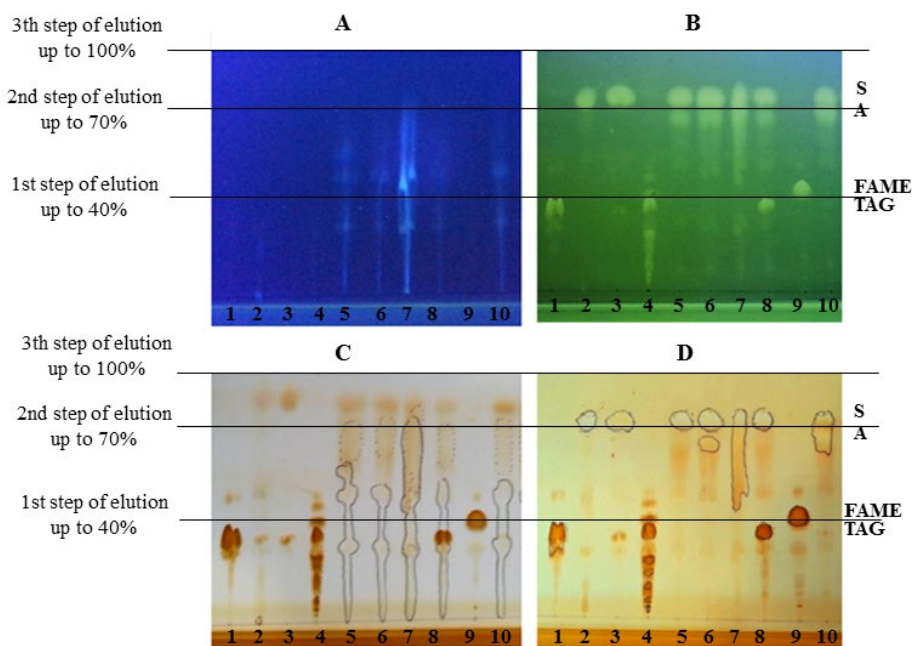


Figure 1. Summary of TLC developed plates (F-254s silica gel) impregnated with berberine sulphate (B and D) using the spraying methodology developed in this work, and non-impregnated – (A and C). Development methodology according to D1 in Table 4; A and B - visualization in 365nm UV light; C and D- Visualization in visible light after exposure of the TLC plate to iodine vapour. Samples (Table 1.) 1- Rapeseed oil, edible, 2-PAO6, 3- motor oil, synthetic poly-alpha-olefin type, commercially available, 4- Lubricating oil of natural origin with a package of additives, commercially available, 5 -SAE10/95, 6-SAE30/ 90, 7-Brightstock, 8- lubricating oil produced on the basis of a mixture of vegetable and mineral oil, 9-FAME, 10- machine oil produced on the basis of crude oil processing.

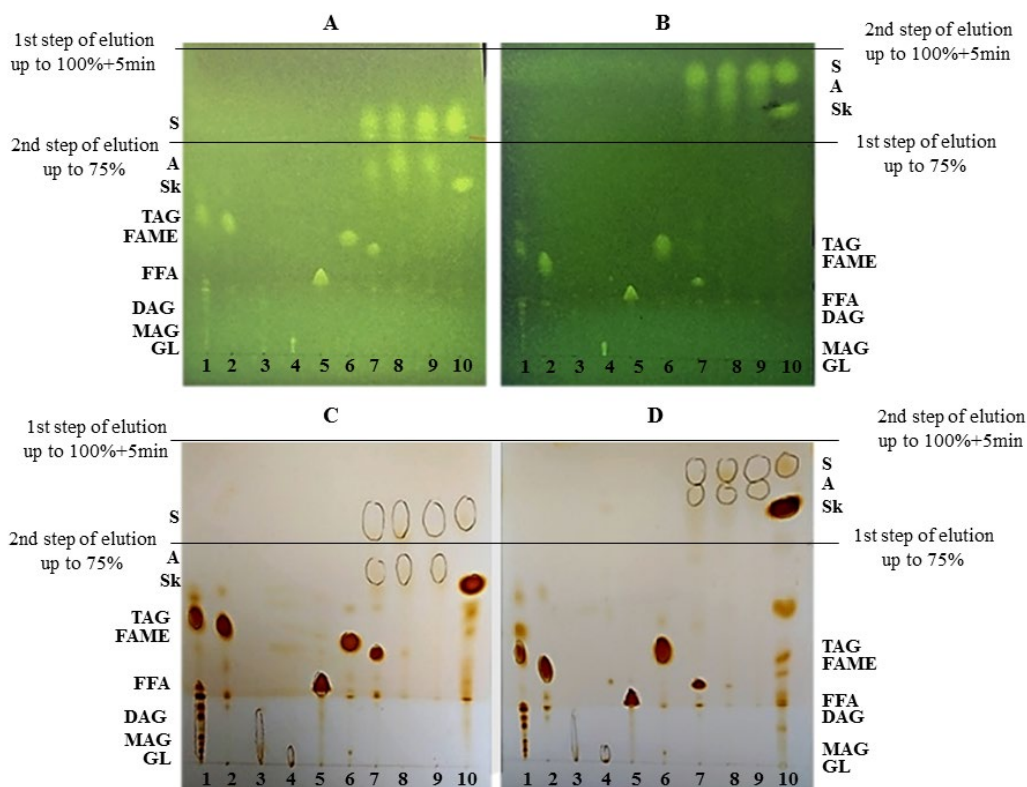


Figure 2. Exemplary photographs of TLC developed plates obtained under development conditions and the separation of the same samples on silica gel plates impregnated with berberine sulphate with the use of different order of the solvent strength sequence of the solvent in successive development steps. Development conditions according to Table 4: A1– plates A, C -

increase in the polarity and solvent strength of the solvent with successive development steps; B1 - plates B, D - decrease in the polarity and solvent strength of the solvent in successive development steps; Visualization: A, B: under 365nm UV light, C, D: under visible light after exposure of the TLC plates to iodine vapour. Samples: 1- mixture - SAE 30/90 base oil, Sq, TAG, DAG, FFA, MAG, glycerol, 2-mixture of sunflower and rapeseed vegetable oil (1:1 v:v), 3-DAG, 4-MAG, 5-FFA, 6-FAME, 7- lubricating oil produced on the basis of a mixture of vegetable and mineral oil, 8- machine oil produced on the basis of petroleum, 9-SAE 30/90, 10-PAO 6 + squalene.

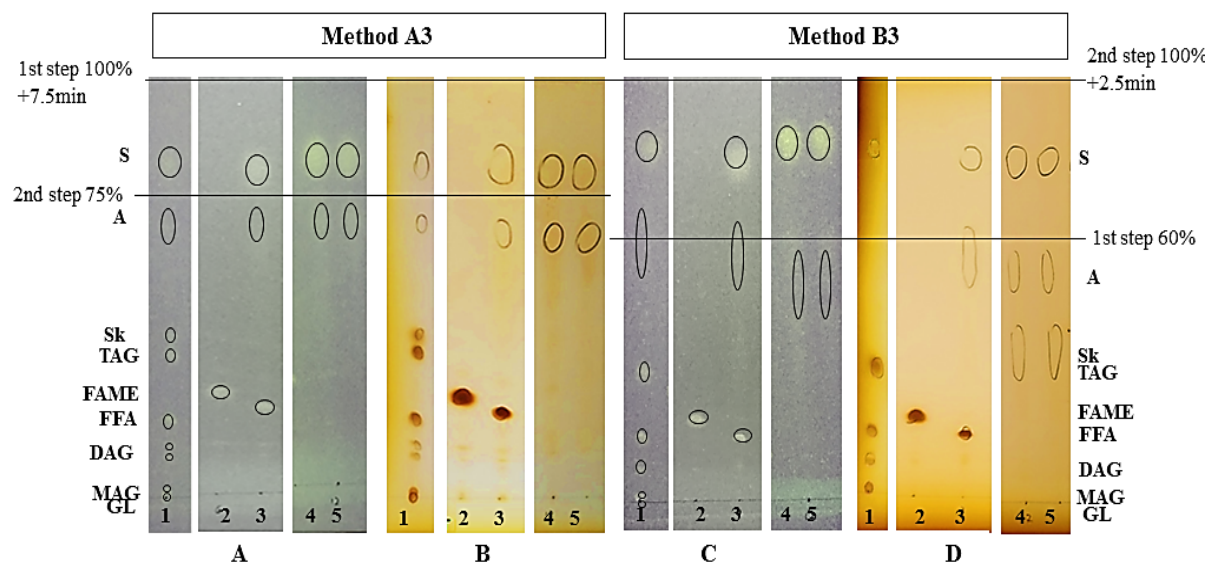


Figure 3. Exemplary photographs of TLC developed plates obtained for two-step development under conditions A3 and B3 ; according to Table 4; separation carried with: increase of solvent polarity and solvent strength in successive development steps (A, B); decrease in the polarity and solvent strength of the solvent in successive development steps (C, D); Visualization: A, C: under UV 365nm light, B, D: under visible light after exposure of the TLC plates to iodine vapour. Samples: 1- mixture - mineral base oil, SAE 30/90, Sq, TAG, DAG, FFA, MAG, GL; 2-FAME, 3- lubricating oil for cutting machines, based on a mixture of plant and mineral (petroleum) oils, 4-SAE 10/ 90, 5-SAE 30/90.

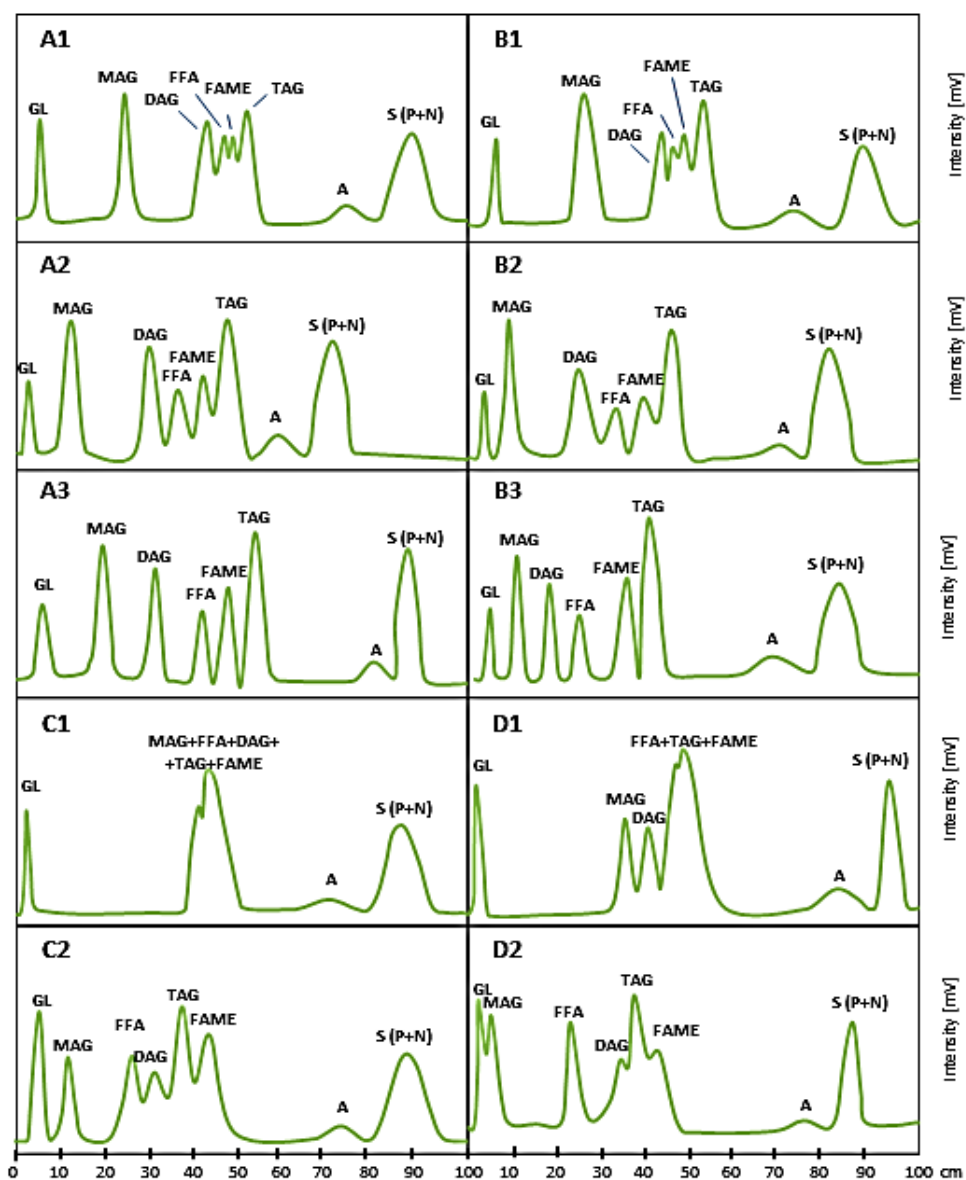


Figure 4. Summary of TLC-FID chromatograms for the A1, A2, A3, B1, B2, B3, C1, C2, D1 and D2 development conditions (according to Table 4) for 0.5 μ L sample volume. Sample concentrations: 1.42 mg/mL GL, 1.42 mg/mL TAG, 2 mg/mL MAG, 1.42 mg/mL DAG, 1.42 mg/mL FFA, 1.42 mg/mL FAME, 1.42 mg/mL S30 (S30 consists of S and A in proportions S:A equal to 3:1 by mass), applied on TLC-FID rods in the form of a solution in a mixture of acetone: dichloromethane 3: 4 (v: v) as spots with minimal width.