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A novel SPME fibre for fatty acid determination

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A novel solid phase microextraction fibre consisting of a sorbent Xiks-1 fixed on a stainless steel support is presented for the extraction of fatty acid derivatives. The sorbent is incorporated into a slot. The sorbent particles do not contact with the outer needle and thus the mechanical damage of the coating is avoided. Fatty acids were derivatized using ethyl chloroformate. The effects of extraction and desorption parameters including the extraction and desorption time, desorption temperature and ionic strength on the extraction/desorption efficiency have been studied. The calibration graphs were linear in the concentration range up to 5×10^{-4} mol L^{-1} ($R^2 > 0.996$). The detection limits were 1.1×10^{-7} mol L^{-1} for palmitic acid, 1×10^{-7} mol L^{-1} for stearic acid and 1×10^{-6} mol L^{-1} for azelaic acid. Repeatabilities of the results were up to 15%. A possibility to apply the proposed method for the identification of oil binders was demonstrated.

Key words: solid phase microextraction, sorbent coating, gas chromatography, fatty acids

INTRODUCTION

The identification of binding media can give valuable information for characterizing the technologies employed by artists facilitate the authentication of the work of art and is helpful to select the appropriate restoration and conservation treatment.

Painting techniques are distinguished according to the kind of binder used. In Europe since the fifteenth century, vegetable oils have gradually replaced proteinaceous binders because oil makes the paint more transparent and less opaque [1]. Not all vegetable oils are suitable for painting, only the ones called the drying oils that are able to dry chemically, i.e. to cross-link to a semi-solid phase upon the exposure to air. Since the medieval times, the most important oils are the oils of linseed, poppy seed and walnut and are frequently used for painting today [2].

The drying oils are made of triglycerides with high proportion of polyunsaturated fatty acids (oleic, linoleic, linolenic acids) and of lower quantity of saturated fatty acids (palmitic, stearic acids). The chemical drying of the oils is a result of auto- and/or photoxidation of unsaturated fatty acids [3]. In the course of time, the contents of the unsaturated acids diminishes and the contents of the formed dicarboxylic acids (pimelic, suberic, azelaic, sebacic) increases [4]. This allows to determine the age of a painting by the contents of the formed dicarboxylic acids (mainly azelaic acid) [1]. On the other hand, the saturated fatty acids undergo little change under aging. As the contents of the saturated fatty acids vary from oil to oil, the identification of different oils is often based on the ratio of palmitic and stearic acids (P/S) [5].

The determination of fatty acids is mostly carried out by gas chromatography. However, fatty acids are polar compounds, and therefore, are of low volatility due to their tendency to self-associate or to adhere to the walls of GC columns or other sorbtive surfaces. Because of this, a preliminary derivatization of fatty acids is required to make them volatile and thereby eligible to GC [6]. In [7, 8], the fatty acid derivatization methods and their advantages and disadvantages have been reviewed. The most frequently used derivatization modes are alkylation and silylation. Although many methods have been developed for the alkylation, they can be divided into three main groups: those using basic catalysis, those using acid catalysis and those combining both catalysisses [9]. Chlorotrimethylsilane is one of the most promising acid catalysts allowing the esterification and transesterification of fatty acids in propanol [9] or pentanol [10]. Alkylchloroformate induced esterification is described in [11, 12]. The use of alkylchloroformate is an attractive approach since derivatization occurs readily in aqueous media at room temperature, resulting in stable and easy-to-handle derivatives.

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The most frequently used silylation reagents are trimethylsilylimidasole [8], bis(trimetylsilyl)trifluoroacetamide [11] and *N*-methyl-*N*-(*tert*-butyldimethylsilyl) trifluoroacetamide [13, 14].

Another approach is thermochemolysis, i.e. heating in the presence of methylammonium reagents. The most popular reagents for fatty acids derivatization are N-tetramethylammonium hydroxide [3, 15], (m-trifluoromethylphenyl)trimethylammonium hydroxide [5] and phenyltrimethylammonium hydroxide [15].

The samples of paintings normally available for analysis are characterised by their small size (<0.1 mg); to avoid further deterioration of the work, therefore, highly sensitive techniques are needed for their identification [16].

Due to simple, solventless and flexible properties of SPME introduced by Pawliszyn and co-workers [17], it has become an attractive alternative to the conventional sampling techniques. Nevertheless, to our knowledge, only one work on SPME for fatty acid analysis applying commercial SPME fibres and on-line methylation with phenyltrimethylammonium hydroxide has been produced [18].

Besides, commercial fused silica fibres are expensive, fragile and extra care must be taken during their use. Therefore, more robust SPME fibres with a long life and low cost are highly desirable.

Till now, several home-made SPME devices on the basis of a mechanically resistant metal wire have been reported. One of the ways of the production of metal core fibres is based on the electrochemical deposition of a coating such as anodized aluminium [19], a potentiometrically deposited Cu (I) on a copper wire [20], or conductive polymers such as polypyrrole, polyaniline and their derivatives as the extraction phases [21, 22].

One more approach is a physical deposition of the coating. In [23] a SPME system consisting of a stainless steel wire coated with commercial silicone-based high-temperature resistant glue was developed. Faraizadeh et al. [24, 25] constructed SPME using alumina powder and polyvinyl chloride dispersed in tetrahydrofuran or activated charcoal and polyvinyl chloride with a piece of silver wire as a fibre support. Lee et al. [26] proposed stainless steel fibres coated with phenyl, C18 and C8 bonded silica particles, normally used as HPLC stationary phases, immobilized using high temperature epoxy glue.

In this study we are presenting a novel SPME fibre coated with a sorbent Xiks-1 fixed on a stainless steel support with a help of high-temperature silicone glue, and investigate the possibility to apply this home-made SPME system for fatty acids determination in binding media.

EXPERIMENTAL

Reagents and solutions

Azelaic acid (≥99%), palmitic acid (≥99%), stearic acid (≥99.5%), ethyl chloroformate (≥98%), pyridine (≥99.5%), nonadecane (≥99%) and hydrochloric acid (≥36.5%) were purchased from Fluka. 2-Butanone (99.5%), *o*-xylene (98%), *n*-pentanol (98%) and *n*-butyl acetate (99%) were purchased from Sigma. Ethanol (GC grade), Na_2SO_4 (analytical grade) and NaCl (analytical grade) were purchased from Reachim (Ukraine). Linseed oil and walnut oil were purchased from "Zavod Chudozhestvenych Krasok" (Russia), and sunflower oil – from "Lukas" (Germany). Silicone glue "Silicone temperature" was purchased from Technicoll (Poland). Sorbent Xiks-1 (anionite, particle size 25–40 μm) was purchased from Chiju Kalur (Estonia). All the reagents were used without a further purification.

A standard stock solution of the analytes (azelaic, palmitic and stearic acids) with a concentration 0.01 mol L^{-1} was prepared in methanol or ethanol. The stock solution was stored refrigerated at +4 °C. Working standard solutions were prepared just before the use by diluting the standard stock solution with methanol or ethanol to the required concentrations.

For the evaluation of the selectivity of the coating, a stock standard solution of 2-butanone, *n*-pentanol, *n*-butyl acetate and *o*-xylene (40 mg L^{-1} of each compound) was prepared in distilled water. A working standard solution containing 5 mg L^{-1} of each compound was prepared just before the use by diluting the stock standard solution with distilled water.

Test painting specimens

A deal board was impregnated with 3% (w/w) of a aqueous solution of fish glue and dried for 2 days. Lead white was dispersed in the linseed oil, walnut oil or sunflower oil and spread in a thin layer on the impregnated deal board. Then the test specimen was dried at room temperature for 7 days.

Instrumentation

Gas chromatography was carried out in a Varian 3400 gas chromatograph equipped with a flame ionization detector coupled with an integrator SP4290 (Spectra-Physics) and fused silica capillary column RTx-CL Pesticides (30 m × 0.53 mm i.d.) (Restek, Bellefonte, PA, USA). The injection port temperature was 260 °C, the detector's temperature was 240 °C, the oven temperature program was initially set to 100 °C for 2 min, then gradually ramped to 120 °C (2 °C/min), to 240 °C (10 °C/min) and held for 10 min. The following gas flow rates were used: carrier (nitrogen) 10, make-up gas 20, hydrogen 30 and air 300 mL min^{-1}.

A SPME fibre holder for manual use (Supelco, St. Louis, MO, USA) was used. SPME was carried out from 1.8 mL of the solution placed in a 2 mL glass vial closed with a Teflon septum containing cap.

Scanning electron microscopy (SEM) was performed on a microscope EVO-50 (Great Britain). The fibre had been sputter-coated with a thin gold film before SEM was carried out (magnification 500× at 20 kV).

Procedures

The sorbent fixing glue was prepared by mixing of 1.0 g of the high-temperature resistant adhesive "Silicone temperature" with 2.0 mL of toluene. The SPME device was modified from a commercial SPME device. The septum-piercing needle was removed and replaced with a larger one. A stainless steel plunger needle (300 μ o.d.) was used as a support for the coating. The end of the plunger needle (2 cm length) was polished so as to get a slot. The plunger needle was mounted inside the external needle, cleaned with acetone in an ultrasonic bath for 10 min and dried at room temperature. A thin layer of the glue solution was spread on the slot of the plunger needle and the sorbent Xiks-1 was gently pressed into the glue. The coated fibre was dried at room temperature in the vertical position for 2 h and then conditioned under nitrogen in the injection port of the gas chromatograph at 300 °C for 1 h.

The binder hydrolysis was based on acid hydroslysis with hydrochloric acid. 1 mg of the sample was placed into the 2 mL screw-capped vial and hydrolysed with 100 μL 6 mol L^{-1} HCl for 8 h at 110 °C in N_2 atmosphere.

RESULTS AND DISCUSSION

Coating selectivity

The surface of the coating was examined by scanning electron microscopy. The slot of the plunger needle coated with the sorbent Xiks-1 particles is evidenced in the photography (Fig. 1).

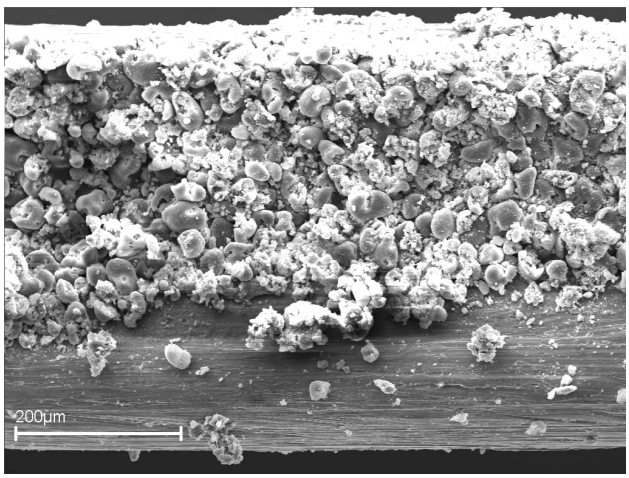

Fig. 1. Scanning electron microscope photography of the SPME fibre coated with the sorbent Xiks-1 (magnification 500× at 20 kV)

For the evaluation of the selectivity, the fibre was tested for four different classes of compounds. For this reason the fibre was held in 10 mL of the aqueous solution containing 5.0 mg L^{-1} of 2-butanone, *n*-pentanol, *n*-butyl acetate and *o*-xylene for 15 minutes. The peak areas were compared with those obtained after a direct syringe injection (1 μL) of the same solution. As illustrated in Fig. 2, the fibre showed good extraction efficiency for *o*-xylene and *n*-butyl acetate. On the other hand, fibre extraction resulted in the decrease of *n*-pentanol and 2-butanone peaks. This indicates that the selectivity of the fibre can be observed for esters and aromatic hydrocarbons in the presence of alcohols and ketones. This fact encouraged us to examine the applicability of the fibre for SPME of ethyl esters obtained after the derivatization of fatty acids using ethyl chloroformate in the presence of ethanol.

Fatty acid derivatization

A lot of fatty acid derivatization reagents based on the silylation and alkylation reactions are suggested. However, it seems that the use of alkylchloroformates is the most promising approach since derivatization occurs readily in aqueous media at room temperature, resulting in stable and easy-to-handle derivatives [11, 12].

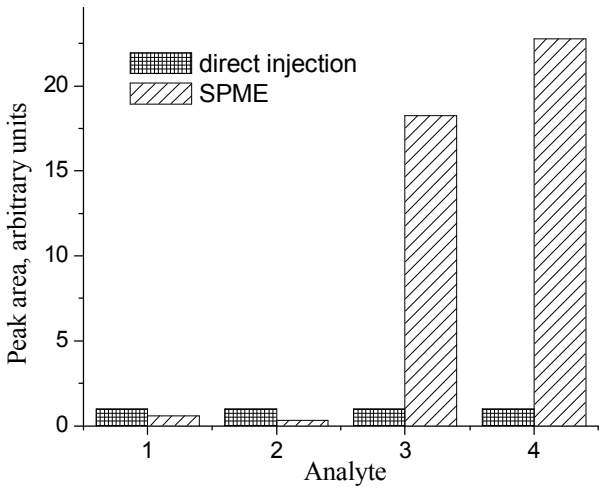

Fig. 2. Comparison of the extraction efficiencies for various compounds obtained by a direct SPME using a stainless steel wire coated with silicone glue. (1) 2-butanone; (2) *n*-pentanol; (3) *n*-butylacetate; (4) *o*-xylene. The concentration of each compound in the sample is 5.0 mg L^{-1}

For the derivatization process ethyl chloroformate [12] and isobutyl chloroformate [11] are described. In order to get derivatives with a lower boiling temperature, and hence with a shorter retention time, we used ethyl chloroformate that in the presence of ethanol and pyridine transformed the fatty acids into ethyl esters.

Standard solutions of fatty acids in methanol and in ethanol were examined. 100 μL of standard solution of fatty acids, 100 μL of ethanol:pyridine (4:1), 10 μL of the solution of internal standard nonadecane and 40 μL of ethyl chloroformate were transferred into the 2 ml screw-capped vial. The mixture was shaken vigorously for 30 s.

In the case of the methanolic solution, 6 peaks corresponding to methyl and ethyl esters of the three examined fatty acids were observed (Fig. 3a). Because of this, the standard solution in methanol was substituted with the standard solution in ethanol. In this case, each

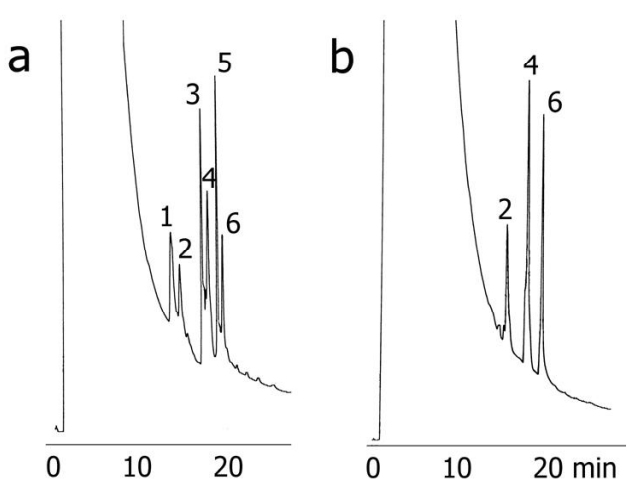

Fig. 3. Chromatograms obtained after the derivatization of fatty acids with ethyl chlorormate in the presence of methanol (*a*) and ethanol (*b*). (1) azelaic acid methyl ester; (2) azelaic acid ethyl ester; (3) palmitic acid methyl ester; (4) palmitic acid ethyl ester; (5) stearic acid methyl ester; (6) stearic acid ethyl ester

fatty acid gave only one derivatization product – ethyl ester (Fig. 3b).

Since the volume of the solution was too small to dip the fibre for SPME, the solution was diluted to 1.8 ml with distilled water before carrying out SMPE.

Optimisation of the desorption parameters

Desorption investigations were accomplished after the direct SPME of fatty acid derivatives. The temperature of the injection port is an important factor in the fibre desorption as well as the coating/gas distribution constants of the adsorbed analytes, rapidly decrease with the temperature increase allowing an efficient desorption of the analytes. On the other hand, desorption temperature is limited by the thermal stability of the coating. There-fore, before the desorption temperature investigations, the thermal stability of the coating was evaluated. Fig. 4 shows blank chromatograms at 260, 280 and 300 °C. It is evident that the coating can be operated without any damage at 260 °C. However, when it was exposed to 280 °C, some artifacts were observed and they increased at 300 °C of injector temperature. So, for the further experiments the desorption temperature of 300 °C was selected.

Desorption time from 1 to 15 min was investigated. The carry-over was measured with one blank injection following the initial desorption. The results showed that at the desorption temperature of 260 °C all the tested compounds were quantitatively desorbed from the fibre coating after 15 min and no carry-over effect was observed in the blank injections. Therefore, in the further work a fifteen-minute desorption time was used.

Extraction parameters

The effect of the main extraction parameters, such as the solution stirring rate, extraction time and salt addition to the sample were studied.

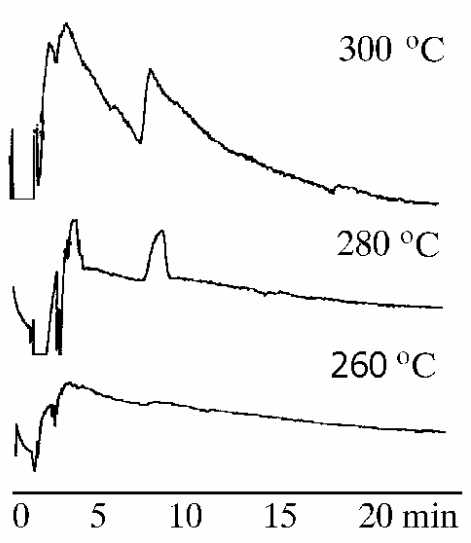

Fig. 4. Blank chromatograms for the Xiks-1 coated SPME fibre desorbed at different temperatures

Stirring of the sample solution reduces the time required to reach the equilibrium and the extraction time by enhancing the diffusion of the analytes towards the fibre. We applied a maximum stirring rate (1500 rpm) that could be reached with a help of the magnetic stirrer we used.

SPME was carried out at room temperature, since at elevated temperatures the coating/water distribution constant of the analytes decreases resulting in the decrease in the extraction efficiency.

For optimum repeatability of the analysis, an equilibrium between the sample and the fibre should be reached. The extraction time was studied in the range of 10–60 min. However, even 60 min were not sufficient to reach the equilibrium (Fig. 5). Because of this fact for the further work we chose non-equilibrium conditions and

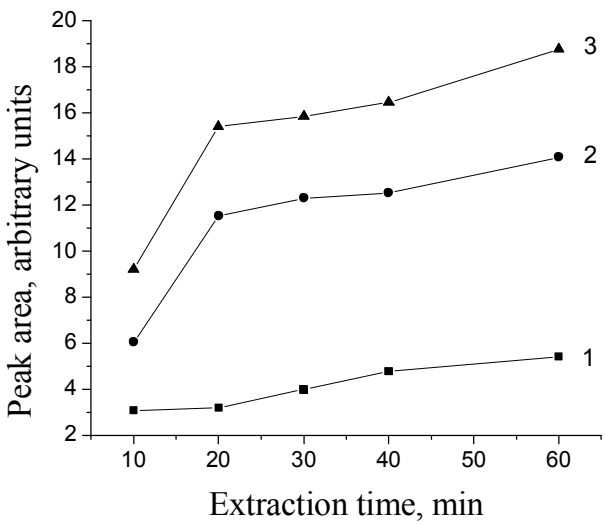

Fig. 5. Effect of the extraction time on the peak area of (1) azelaic acid ethyl ester, (2) palmitic acid ethyl ester, (3) stearic acid ethyl ester. Desorption at 260 °C for 15 min

established a thirty-minute extraction time always maintaining the extraction time precisely the same.

The addition of a salt to an aqueous sample solution generally causes a decrease in the solubility of the organic compounds in water. This has been widely used to enhance the extraction of analytes. In order to increase the ionic strength of the solution, for the dilution of the derivatized solution we used a saturated solution of NaCl or Na_2SO_4 instead of distilled water. In both cases, the extraction efficiency increased in comparison with the extraction carried out from the solutions diluted with distilled water. However, a more significant increase (about 1.5 times) was observed in the presence of Na_2SO_4 solution. This is in accordance with the ionic strength of the solutions that in the case of Na_2SO_4 is about 1.8 times higher then for NaCl solution. In further experiments, a saturated Na_2SO_4 solution was used for the dilution of the derivate.

Analytical performance

The quality parameters of the SPME method such as linearity, limits of detection and repeatability were measured under the optimised conditions described above. The calibration graph was linear from 1.3×10^{-6} mol L^{-1} up to 5×10^{-4} mol L^{-1} for azelaic acid ethyl ester and from 1.2×10^{-7} mol L^{-1} up to 1.3×10^{-3} mol L^{-1} for palmitic and stearic acis ethyl esters. The correlation coefficients were: for azelaic acid ethyl ester 0.996 (n = 8), for palmitic and stearic acid ethyl esters 0.998 (n = 11). The limits of detection, defined as three times of baseline noise are presented in Table 1.

In order to assess the repeatability, five repeated measurements at two different concentrations of the analytes were carried out. The RSDs have been calculated and are summarized in Table 2. These data show that the repeatability of the method is satisfactory.

Table 1. **Fatty acid detection limits, mol L^{-1}**

Analyte	Detection limit, mol L^{-1}
Azelaic acid	1.0×10^{-6}
Palmitic acid	1.1×10^{-7}
Stearic acid	1.0×10^{-7}

Table 2. **Repeatabilities of the SPME of fatty acids (n = 5)**

Analyte	Concentration, mol L^{-1}	RSD, %
Azelaic acid	2.8×10^{-4}	12,3
	1.4×10^{-5}	14.2
Palmitic acid	2.8×10^{-4}	7.8
	1.4×10^{-5}	11.7
Stearic acid	2.8×10^{-4}	9.8
	1.4×10^{-5}	9.6

Test painting specimen analysis

A possibility to use the fibre for a real sample analysis was demonstrated by applying it to the determination of fatty acids in test painting specimens.

As it was mentioned in the Introduction, the identification of lipid binders is based on the ratio of palmitic and stearic acids, and the quantity of azelaic acid is related to the age of a painting.

Prior to the derivatization with ethyl chloroformate we employed acid hydrolysis.

For the analysis, the paint layer was mechanically separated from the board using a scalpel. 1 mg of the sample was weighted, 10 μL of the internal standard solution was added, the sample was hydrolysed, derivatized and extracted using the SPME fibre as described above. In parallel, linseed oil, walnut oil and sunflower oil were analysed following the same procedures.

Azelaic acid was not detected probably because it was not initially present in the oil and formed under aging. The painting specimens we analysed were rather fresh so the content of azelaic acid was under the detection limit. The ratios of palmitic and stearic acids are presented in Table 3. There is quite a good agreement between the ratios in test painting specimen, corresponding oil and the data presented in [27] indicating that the suggested method can be successfully applied for oil binder analysis in real art works.

CONCLUSIONS

The novel SPME fibre consisting of the sorbent Xiks-1 fixed on the stainless steel support is an attractive alternative to commercially available silica fibres. Since the sorbent is incorporated into a slot, the sorbent particles do not contact with the outer needle and thus the mechanical damage of the coating is avoided. The proposed fibre selectively extracts esters in the presence of alcohols, therefore, after the derivatization procedure using ethyl chloroformate, the fibre was adapted for the determination of fatty acids in the form of their ethyl esters. However, together with the fatty acid derivatives the coating extracts pyridine that is present in the solution resulting in a big pyridine peak in the chromatogram. In order to achieve an enhanced selectivity towards the target analytes the studies on substituting the sorbent Xiks-1 with another sorbent are underway.

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Table 3. **Relative amount of palmitic and stearic acids in oil, test painting specimen and according to the literature**

Oil	P/S in oil	P/S in paint specimen	P/S in oil according to [27]
Linseed	2.25	2.30	1.2–2.3
Walnut	2.73	2.83	2.2–3.2
Sunflower	2.46	2.49	2.7

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NAUJAS KIETAFAZĖS MIKROEKSTRAKCIJOS STRYPELIS RIEBALŲ RŪGŠTIMS NUSTATYTI

S a n t r a u k a

Riebalų rūgščių derivatizacijos produktams nustatyti pasiūlytas naujas kietafazės mikroekstrakcijos strypelis, pagamintas priklijavus sorbentą Xiks-1 nerūdijančio plieno adatos griovelyje. Sorbento dalelės nekontaktuoja su išorine adata, todėl danga nenusitrina. Riebalų rūgštys derivatizuotos panaudojus etilo chloroformiatą. Ištirti ekstrakciją bei dujų chromatografinį nustatymą sąlygojantys parametrai (desorbcijos temperatūra ir trukmė, ekstrakcijos trukmė, tirpalo joninė jėga). Tiesiniai koncentracijų intervalai buvo iki 5×10^{-4} mol L^{-1} ($R^2 > 0,996$). Aptikimo ribos buvo $1,1\times10^{-7}$ mol L^{-1} palmitino rūgščiai, 1×10^{-7} mol L^{-1} stearino rūgščiai ir 1×10^{-6} mol L^{-1} azelaino rūgščiai. Rezultatų pasikartojamumas buvo iki 15%. Parodyta galimybė pritaikyti pasiūlytą metodą aliejiniams rišikliams identifikuoti.