

Analysis of free fatty acids in soap samples by means of gas chromatography-mass spectrometry

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In this work a new, accurate and reliable method, which has been applied to determine the quantitative and qualitative composition of free fatty acids in samples of laundry and toilet soap, is described. Silylation as a derivatization method for analysis of free fatty acids in soap samples by gas chromatography-mass spectrometry, using ammonium sulfate as a catalyst, a low-cost silylating agent hexamethyldisilazane (HMDS) and chloroform as a solvent, was evaluated. Gas chromatography-mass spectrometry analysis was carried out using a DB-5 capillary column. The method was validated for linearity, precision and limits of detection and quantification. It was found that the validated method had an acceptable linearity with the determination coefficient >0.999 within the concentration range 0.005–2 mg/ml. The limits of detection and quantification were obtained from the regressive analysis and were 2.5 and 8 $\mu\text{g/ml}$, respectively. The validated method was applied for analysis of four samples of different quality soaps. The determined amount of free fatty acids in the soaps was ranging between 1.24 and 5.09%. This method can be applied to the analysis of the composition of free fatty acids in different soap samples.

Key words: free fatty acids, soap, silylation, GC-MS, Folch et al. method

INTRODUCTION

Fatty acids (FAs) and free fatty acids (FFAs) are non-volatile aliphatic monocarboxylic compounds, one of the most important constituents of fats and also can be determined as a base of soap. Widely found in nature and well-defined FFAs and FAs have been studied extensively for their interesting physical properties, trying to find the fastest and the most accurate methods to identify and measure these compounds in different matrices (vegetable oil, fuel, bacteria, leaves, eggs) [1]. However, no data on identification and determination of FFAs by gas chromatography-mass spectrometry (GC-MS) in laundry and toilet soaps has been published yet. On the other hand, there are some methods, which are

applied to determine total fatty acids, neutral oil, volatiles, moisture and residual solvent [2], metals [3] in detergents.

Soap is one of the most popular detergents in personal care. It is produced via the saponification reaction (hydrolysis) of FAs triglycerides with a strong base (usually potassium or sodium hydroxides), producing soap (potassium or sodium salts of FAs) and glycerol.

Soap quality depends on the composition of saponified FAs, i. e. saturated FAs give light open foam bubbles and solid, hard consistency, while unsaturated FAs provide moisturizing, conditioning and nourishing properties [4]. Although salts of FAs are the main component in soap, there are other components – glycerol, FFAs, unsaponifiable matter (sterols, higher aliphatic alcohols, pigments, hydrocarbons, etc.). FFAs, which are in a free form, also play an important role in soap quality – presence of these compounds indicates

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higher quality of a detergent. Soap with FFAs is richer in moisturizing and high-fattening, gives better foaming [4, 5] – these characteristics are the quality soap indicators.

FFAs content can vary according to the fats and oils source. Usually, laundry detergents, which are of lower quality, are made of fats of animal origin. Vegetable oils, such as palm and palm kernel oils, are often used to manufacture toilet soaps with improved compositions (with essential oils, herbal extracts additives, etc.). Manufacturers can use animal origin fats for the production of toilet soaps as well. Animal fats and vegetable oils differ in their composition. Vegetable oils are rich in monounsaturated and polyunsaturated FAs, while animal fats are mainly composed of saturated FAs [6, 7].

As soap has a complex nature, its analysis is a challenging problem. Extracted FFAs are non-volatile compounds with high polarity. Derivatization is necessary to increase the volatility, thermal stability and improve gas chromatographic analysis characteristics of analytes by decreasing their polarity. A broad range of derivatization procedures is used for this purpose, namely alkylation, acylation and silylation [8]. The silylation reaction converts the hydroxyl groups into their corresponding trimethylsilyl-derivatives via a substitution reaction. This method is quite simple and fast, silylation reagents are generally cheap, non-toxic and widely available. However, the silylation presents some disadvantages – the reaction is moisture sensitive and some precipitate can be produced (it depends on silylation reagent which is used for derivatization) [9]. To our knowledge, this is the first study where silylation is applied to improve the determination of FFAs in soaps.

The aim of the research was to apply extraction and silylation as a derivatization method and a GC-MS method to determine FFAs as one of the quality parameters in soaps and carry out the necessary method validation steps.

EXPERIMENTAL

Samples and reagents

Four samples of laundry and toilet soaps – laundry soap Stumbras, toilet soaps Elegance, Galant and Smilė were donated by Naujoji Ringuva (Panevėžys, Lithuania). Samples were stored at room temperature in a dry, dark place until use. Ammonium sulfate ($\geq 99.0\%$) and methanol (99.9%, HPLC) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hexamethyldisilazane ($(\text{CH}_3)_3\text{SiNHSi}(\text{CH}_3)_3$, $\geq 99.5\%$, GC) used as a silylation agent and an octadecanoic acid standard ($\geq 99.5\%$) used as an internal standard were obtained from Supelco (St. Louis, USA). Chloroform and trifluoroacetic acid (TFA) for synthesis were purchased from Lachema (Brno, Czech Republic) and Merck (Darmstadt, Germany), respectively.

Instrumentation

GC-MS analyses were performed using a model GC-2010 gas chromatograph equipped with a model GCMS-QP2010 mass

spectrometer and a model AOC-20i autoinjector (Shimadzu, Japan). Separation of FFAs was carried out using a low bleed capillary column DB-5ms (30 m \times 0.25 mm \times 0.25 mm) coated with non-polar 5% phenylmethyl- and 95% dimethylpolysiloxane stationary phase from Restek, USA.

Operation conditions were as follows: the injector and detector temperatures were set at 250 and 260 °C, respectively. Samples were injected manually with a split ratio of 1:10. The temperature program was as follows: initial temperature at 50 °C was held for 2 min, increased at 10 °C/min until 180 °C, held for 5 min, increased at 5 °C/min until 280 °C and held for 2 min. Helium was used as the carrier gas, with a linear velocity of 15.1 ml/min, column head pressure: 60.8 kPa.

Soaps FFAs were identified using a mass spectrometer (MS). MS was operated in the electron ionization mode and mass spectra were collected within m/z from 45 to 500.

Experimental conditions

Extraction

FFAs were extracted by a traditional procedure of extraction proposed by Folch et al. [10]. 1 g of the soap sample was weighted into a volumetric flask. The sample was extracted with a 2:1 mixture of CHCl_3 and CH_3OH (v/v) adding it to a final volume of 20 ml. The mixture was treated for ca. 15 min in an ultrasonic bath, the homogenate was filtered through 0.8 μm filter paper to recover the liquid phase. The solvent was washed with 4 ml of 0.88% KCl aqueous solution to remove non-lipid material. The mixture was centrifuged at low speed (2000 rpm, 5 minutes) to separate two phases. Extracted FFAs were dissolved in lower liquid phase – chloroform. All steps were performed at room temperature.

Derivatization

Silylation of FFAs using hexamethyldisilazane (HMDS) was performed following the procedures reported in detail elsewhere [9, 11]. 100 μl of lower liquid phase after extraction were transferred into a 2.5 ml sealed screw cap vial adding 1.5 ml of chloroform as a solvent, 0.025 g of ammonium sulfate as a catalyst and 1 ml of hexamethyldisilazane as a derivatization agent. Samples were heated for 45 min at 60 °C temperature and cooled to room temperature before gas chromatography. All samples were analysed within 12 h since the derivatization.

RESULTS AND DISCUSSION

Method optimization

Most methods for FAs and FFAs analysis in different samples are applied to measure a composition of total FAs and triglycerides [9, 12]. These analytes require high temperature to elute – temperature of methods should be set above 360 °C. As soap is a salt of FAs, FFAs and glycerol without triglycerides in the composition, it is possible to reduce the temperature and use thermal gradient elution for FFAs analysis. This step was carried out before validating the method.

To speed up the silylation reaction a catalyst is necessary. Different catalysts can be used for silylation, namely trifluoroacetic acid, LiClO_4 , $\text{Al}_2\text{O}_3/\text{KF}$, HCl and ammonium sulfate [11]. The most popular combination – trifluoroacetic acid as a catalyst and hexamethyldisilazane as a derivatization agent in this study was not suitable as it resulted in a white precipitate and very low peak areas in the GC-MS chromatogram of the filtrate. This problem was solved selecting ammonium sulfate as the catalyst. As the main FFAs in chromatogram after this change evolved with considerably higher peak areas, no further optimization of the method was required.

Method validation

Linearity

The linearity of peak area response versus concentration for octadecanoic acid was studied within the concentration range of 0.005–2 mg/ml. The constructed calibration curve was evaluated by its correlation coefficient. The calibration equation from three replicate experiments, $y = 7\,390\,483.46 \cdot x$ ($R^2 = 0.9994$), demonstrated good linearity of the method.

Precision

The precision of the analytical method was determined by repeatability (within-day) and intermediate precision (between-day). 1.0 mg/ml concentration of octadecanoic acid was analysed five times in one day for within-day precision and once daily for between-day precision determinations.

The relative standard deviation (RSD) values for within-day precision evaluation (retention time and peak area) were 0.007 and 4.83%, respectively. The values for between-day precision (retention time and peak area) were 0.028 and 65.43%, respectively. Value of RSD for peak area (precision for between-day) is not in an acceptable range. This is due to an unstable derivatized sample. Therefore it was decided to analyze silylated analytes by GC-MS within 12 hours of preparation. This problem is known in literature [9].

Limits of quantitation and detection

Limits of quantitation (LOQ) and detection (LOD) of the method were calculated according to literature [13]. LOQ was determined as the signal to noise ratio of 10:1 and LOD was determined as the signal to noise ratio of 3.3:1 of the averaged value of the octadecanoic acid peak obtained during precision determination experiments. The calculated value for LOQ was 8 $\mu\text{g}/\text{ml}$ and for LOD it was 2.5 $\mu\text{g}/\text{ml}$. The obtained results above proved that the method is sensitive enough for its purpose.

Sample analysis

The validated method of determination of FFAs in soaps was applied for the analysis of laundry and toilet samples in the Lithuanian market – laundry soap Stumbras, toilet soaps Elegance, Galant and Smilė. Figures 1 and 2 report the FFAs qualitative composition of analysed laundry and toilet soaps, named Stumbras and Smilė, respectively. For each sample FFAs composition was analysed in triplicate.

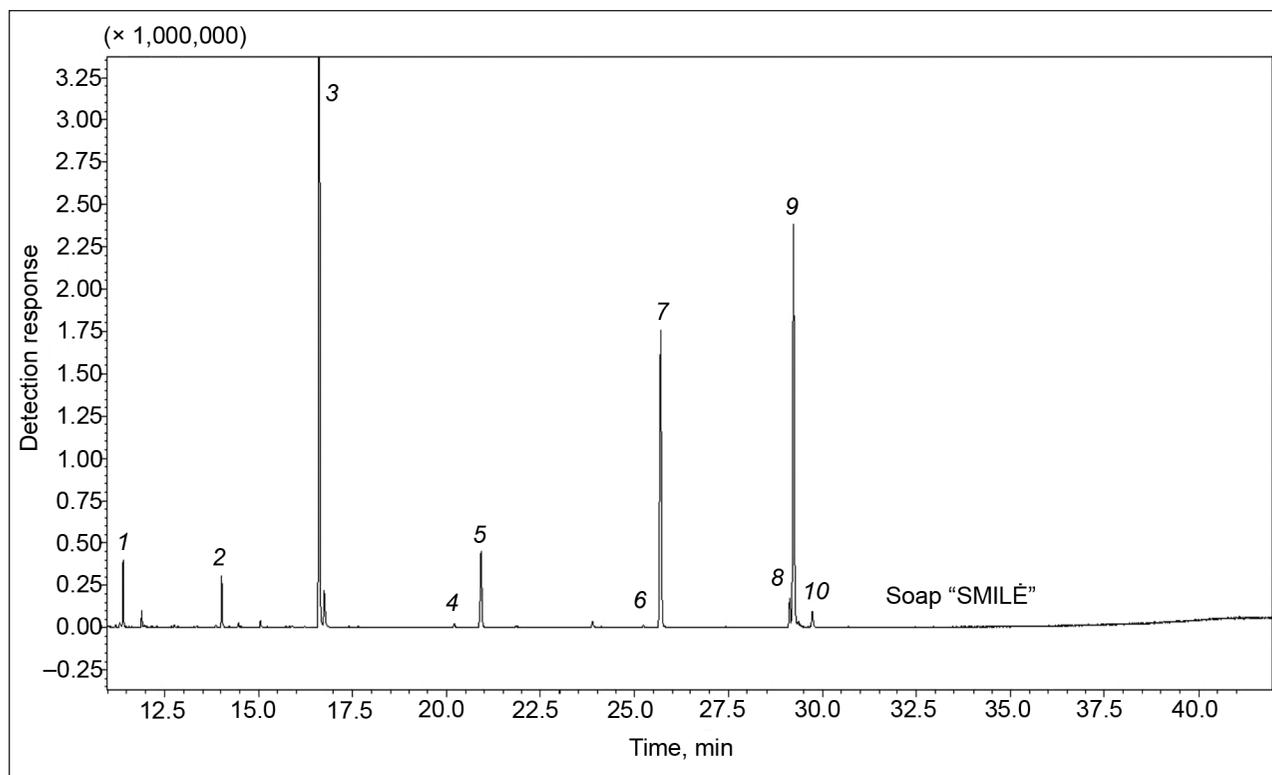


Fig. 1. The composition of TMS FFAs in Smilė toilet soap

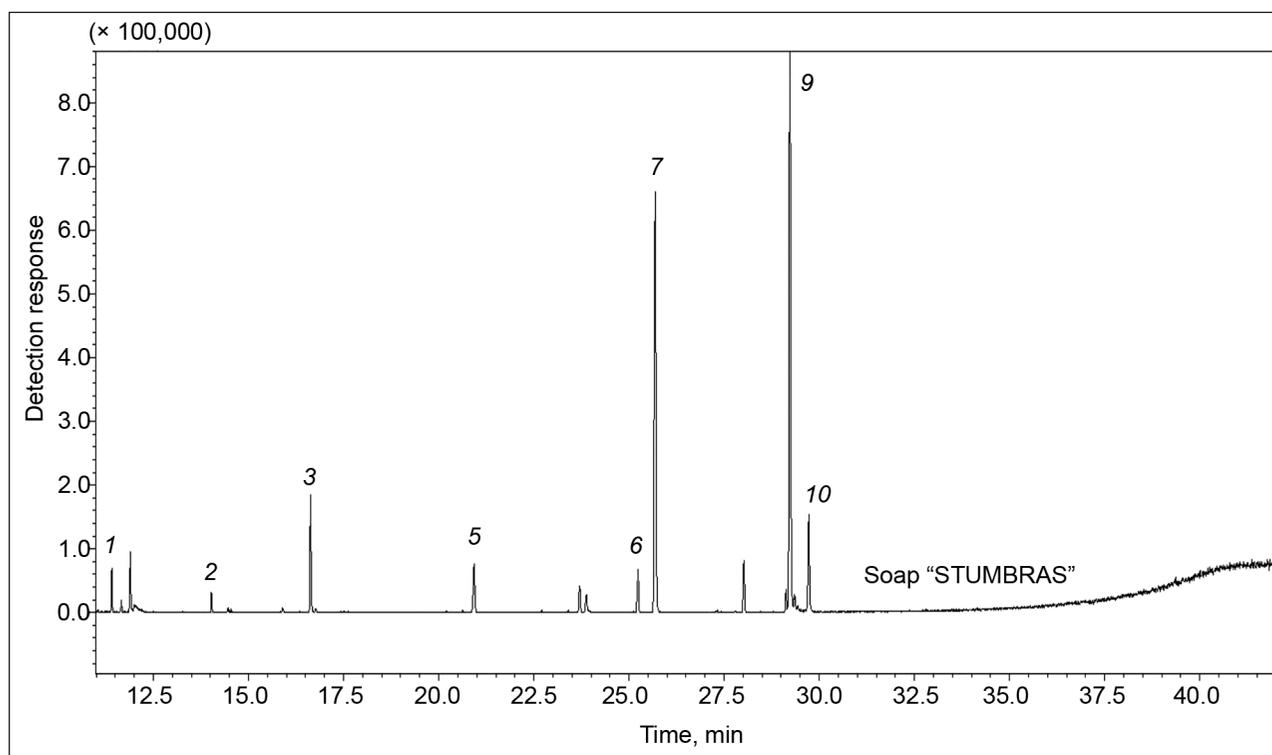


Fig. 2. The composition of TMS FFAs in Stumbras laundry soap

Determined FFAs, containing from 8 to 18 carbon atoms in chain, were (1) trimethylsilyl- (TMS) octanoic acid, (2) TMS decanoic acid, (3) TMS dodecanoic acid, (4) TMS *cis*-9-tetradecenoic acid, (5) TMS tetradecanoic acid, (6) TMS *cis*-9-hexadecenoic acid, (7) TMS hexadecanoic acid, (8) TMS 9,12-octadecadienoic acid, (9) TMS *cis*-9-octadecenoic acid and (10) TMS octadecanoic acid.

It was found that concentration of TMS FFAs in every soap sample was different (Table 1). Results showed that the most common FFAs in all 4 analysed samples were dodecanoic acid, hexadecanoic acid and *cis*-9-octadecenoic acid. For a better analysis of results, Table 1 was converted to Table 2, where TMS FFAs were recalculated to pure FFAs. FFAs quantities in the composition of soaps vary from 1.24 to 5.09% (Table 2). These quantities are within the limits of

acceptable variability for the analyte concentration of this kind of samples [14]. The maximum concentration of FFAs was found in the most expensive Smilė toilet soap as according to the manufacturer it was made from vegetable oils, also its composition was enriched with castor oil (rich in FFAs). As laundry soaps usually have lower quality requirements, the minimal quantities of these analytes were determined in this kind of soap (laundry soap Stumbras). Such low quantities of FFAs show that in manufacturing of these laundry soaps animal fats poor in FFAs were used. In other two economical, moderately priced toilet soaps (Elegance and Galant) there were 3.96 and 4.08% of FFAs, respectively. Results show that these soaps are based on both animal fats and vegetable oils sources. The obtained results of analysis of FFAs in soap samples by GC-MS were approved by the manufacturer.

Table 1. TMS FFAs composition and concentration in samples

Analyte	R _f , min	The name of soap			
		Quantity, mg/g ± SD, %, n = 3			
		Stumbras	Elegance	Galant	Smilė
TMS octanoic acid	11.40	0.16 ± 0.01	4.70 ± 0.02	6.67 ± 0.01	0.95 ± 0.03
TMS decanoic acid	14.02	0.08 ± 0.02	2.84 ± 0.09	3.63 ± 0.24	0.84 ± 0.07
TMS dodecanoic acid	16.62	0.66 ± 0.03	13.98 ± 0.50	15.71 ± 0.35	30.43 ± 0.45
TMS <i>cis</i> -9-tetradecenoic acid	20.63	–	0.58 ± 0.03	0.44 ± 0.33	0.01 ± 0.01
TMS tetradecanoic acid	20.92	0.50 ± 0.03	5.43 ± 0.01	6.00 ± 0.38	2.99 ± 0.04
TMS <i>cis</i> -9-hexadecenoic acid	25.23	0.38 ± 0.02	1.83 ± 0.02	1.09 ± 0.36	0.10 ± 0.01
TMS hexadecanoic acid	25.68	4.63 ± 0.01	11.49 ± 0.23	13.24 ± 0.21	12.45 ± 0.23
TMS 9,12-octadecadienoic acid	29.13	–	–	–	0.98 ± 0.03
TMS <i>cis</i> -9-octadecenoic acid	29.22	8.03 ± 0.04	10.63 ± 0.13	6.17 ± 0.22	17.41 ± 0.56
TMS octadecanoic acid	29.72	1.38 ± 0.16	1.04 ± 0.02	1.59 ± 0.11	0.69 ± 0.09

Table 2. FFAs composition and concentration in samples

Total quantity of FFAs	The name of soap			
	Quantity, %			
	Laundry soap	Toilet soap	Toilet soap	Toilet soap
	Stumbras	Elegance	Galant	Smilė
Saturated FFAs	0.57	2.93	3.47	3.61
Monounsaturated FFAs	0.67	1.03	0.61	1.39
Polyunsaturated FFAs	–	–	–	0.07
Total FFAs	1.24	3.96	4.08	5.09

CONCLUSIONS

The present work describes the validation and application of a new procedure combining extraction with organic solvents, silylation with hexamethyldisilazane and GC-MS for FFAs analysis in soaps. The method was validated for the following parameters: the linearity of the method was acceptable with the determination coefficient ($R^2 = 0.9994$), the precision of the method was evaluated calculating RSD for peak area and retention time for octadecanoic acid, the LOQ and LOD were found by regression analysis, 8 $\mu\text{g/ml}$ and 2.5 $\mu\text{g/ml}$, respectively. The validated method was applied to the determination of FFAs composition in soaps. FFAs quantities in the composition of soaps varied from 1.24 to 5.09%. The maximum concentration of FFAs was evaluated in the most expensive soap, while the lowest concentration was determined in the laundry soap, which is low-priced and commonly has the lowest quality requirements.

This simple, rapid, sensitive, reliable, specific and precise GC-MS method is suitable for determination of FFAs composition in different samples of soaps.

Received 10 June 2013

Accepted 31 July 2013

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DUJŲ CHROMATOGRAFINĖ-MASIŲ SPEKTROMETRINĖ LAISVŲJŲ RIEBALŲ RŪGŠČIŲ ANALIZĖ MUILO BANDINIULOSE

Santrauka

Riebalų rūgštys – alifatiniai monokarboksiliniai lipidų klasės junginiai, plačiai sutinkami gamtoje kaip viena iš riebalų sudėtinųjų dalių. Riebalus veikiant šarmu susidaro muilas – riebalų rūgščių ir šarmo druskos, pasižyminčios plaunančiosiomis savybėmis.

Kokybiško muilo gamybos procese itin svarbus muile esantis sumuilitų ir laisvųjų riebalų rūgščių skaičius, jų įvairovė, sotumas. Šiems junginiams esant nelakiems ir itin poliškiems, junginių analizė tampa sudėtinga užduotimi. Darbe aptariamas ir pritaikomas vienas iš galimų laisvųjų riebalų rūgščių derivatizacijos metodų, taip sumažinant analičių poliškumą ir padarant jas tinkamomis dujų chromatografinėi-masių spektrometrinei analizei. Derivatizacijos reakcija vykdoma naudojant derivatizacijos agentą heksametil-disilazaną, katalizatorių amonio sulfatą ir tirpiklį chloroformą. Susidarę lakūs riebalų rūgščių sililinti junginiai analizuojami įteintu dujų chromatografinės-masių spektrometrinės analizės metodu. Atlikti šie metodo įteisinimo žingsniai – nustatytas analizės metodo kalibracinio grafiko tiesiškumas, glaudumas, specifiskumas, rastos aptikimo ir nustatymo ribos. Įteintu metodu atlikta kokybinė ir kiekybinė laisvųjų riebalų rūgščių analizė keturiuose skirtinguose muilo bandiniuose siekiant nustatyti vieną iš galimų muilo kokybinių rodiklių. Laisvųjų riebalų rūgščių kiekiai muilo mėginiuose kito nuo 1,24 iki 5,09 %.