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Development and validation of chromatography methods for targeted applications

DOCTORAL DISSERTATION

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Audrius
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LIST OF ABBREVIATIONS

APCI – atmospheric pressure chemical ionization
API – atmospheric pressure ionisation
APPI – atmospheric pressure photo ionization
CNT – carbon nanotubes
DSS – dioctyl sulfosuccinate sodium
ECD – electron capture detector
ESI – electrospray ioanisation
GC – gas chromatography
GFC – gel filtration chromatography
GPC – gel permeation chromatography
GCMS – gas chromatography mass spectrometry
HPLC – high performance liquid chromatography
HIC – hydrophobic interaction chromatography
HS – head space
HILIC – hydrophilic interaction liquid chromatography
IPC – hydrophobic interaction chromatography
ID – inner diameter
ICS – tris[3-(trimethoxysilyl)propyl]isocyanurate
LOD – limit of detection
LOQ – limit of quantification
LSV – linear-scan voltammetry
OD – outer diameter
NARP – nonaqueous reversed-phase chromatography
NPC – normal-phase chromatography
MALDI – matrix-assisted laser desorption
MRM – multiple reaction monitoring
MS-MS – quadrupole – quadrupole mass spectrometry
MSPD – matrix solid-phase dispersion
MTBE – methyl tert-butyl ether
PDMS – polydimethylsiloxane
PEG – polyethylene glycol
PLOT – porous layer open tubular
PPG – polypropylene glycol
PTFE – polytetrafluoroethylene
PTI – proton transfer ionization
Q-TOF – quadrupole – time of light mass spectrometry
RP – reverse phase

SB 35 – fuel dye solvent blue 35
SCOT – support coated open tubular
SDME – single drop micro extraction
SEM – scaning electron microscopy
SIFTI – selected-ion flow-tube ionization
SIM – selected ion monitoring
SPCE – flat printed carbon electrode
SPME – solid phase micro extraction
SR 19 – fuel dye solvent red 19
SWV – square-wave voltammetry
SY 124 – fuel dye solvent yellow 124
TOF-TOF – time of light – time of light mass spectrometry
UHPLC – ultra high performance liquid chromatography
UV – ultra violet
UV-VIS – Ultraviolet and visible light
WCOT – wall coated open tubular

1. INTRODUCTION

Because of its simplicity, sensitivity, and effectiveness in separating components of mixtures, gas chromatography is one of the most important tools in analytical chemistry [1]. It is widely used for quantitative and qualitative analysis of mixtures and for the purification of compounds. Gas chromatography is also used to monitor industrial processes automatically [2, 3]. Many routine analyses are performed rapidly in medical and other fields. Gas chromatography is also useful in the analysis of air pollutants, alcohol in blood, essential oils, and food products [4-8].

To assess the performance of capillary columns for gas chromatography, quality control test probes are used. These probes ensure that the columns have been properly deactivated, contain the correct amount of the stationary phase, and have the same relative retention as the last column purchased [9–11]. For column assessment a test mixture composed of various classes of organic components including hydrocarbons, fatty acid methyl esters, acids, bases and alcohols has been proposed [12]. This procedure with subsequent refinements became the benchmark for column testing [13–15]. In the tests, inert probes serve to calculate chromatographic efficiency and as indicators of the efficacy of the injection process. Any tailing or lost response of the acidic probe indicates that the column is basic in nature. Poor peak behaviour of the base indicates that the column is acidic. The alcohol will give an indication if there is any oxygen damage or if there are any exposed silanols. If the peak shapes for all of these compounds are symmetrical, then the column is considered to be inert towards them [9]. The choice of the test probes can either highlight or mask the deficiencies of the column. Ultimately, in order to reveal the drawbacks of the columns, in test mixtures more demanding compounds are often used. As a rule, the compounds have less sterically hindered active groups, have lower boiling points and thus are eluted at lower temperatures [9].

The majority of gas chromatographic research are performed using a temperature gradient of the oven in which the analytical column is placed. The rate and accuracy of the temperature gradient change is an essential factor how the movement of analytes through the column can be controlled. Since the development of gas chromatography as a method, an analytical column has been placed in a closed container or otherwise oven equipped with a heating element, temperature sensor, fan and other components designed to maintain the desired temperature and ensure its stability. All these devices form a rather large structure with a relatively high heat capacity. High heat capacity also

results in high inertia which means that the rate of temperature change is limited to several dozen degrees per minute, what is not always enough to make fast and comprehensive analysis.

In order to speed up the analysis it is necessary to reduce the heat capacity of gas chromatography system. One of the way to do it is to change column shell material from curently generally accepted, because of its inertness, fused silica to conductive material like stainless steel. Stainless steel has quite high resistance to electricity and tubing formed from this material could be used not only as a frame for the column but also serve as a heater.

There are many different types of liquid chromatography techniques and systems available for a wide range of applications all of which are defined as High Performance Liquid Chromatography (HPLC) [16]. HPLC is an analytical technique used to separate, identify or quantify each component in a mixture. The HPLC has developed into a universally applicable method so that it finds its use in almost all areas of chemistry, biochemistry, and pharmacy, such as analysis of drugs, synthetic polymers, pollutants in environmental analytics, determination of drugs in biological matrices, isolation of valuable products, product purity and quality control of industrial products and fine chemicals, separation and purification of biopolymers such as enzymes or nucleic acids, water purification, pre-concentration of trace components and other [17-21].

Fuel dyes provide colour to fuel, and could be identified visually. Fuel dyes can also be used as some fuel markers; they must be of different colour and exact concentration that can be determined by analytical methods. Fuel dyes are used to differentiate fuel with differently applied taxes. Dyes are added to fuel which is used for heating in agriculture, aquaculture and commercial fishing in inland waters. Also, without dyes there are fuel markers which are used to mark the fuel. Fuel markers are the same materials as fuel dyes can change fuel colour, but must be in an exact concentration and could be determined with analytical methods. Fuel dying and marking must be carried out using a specific methodology [22]. However, an exact chemical analysis method which allows the determination of various fuel dyes, markers and, also, their concentration effectively in one run is not yet available. The difference and variety of analytical methods suitable for the determination of fuel dyes and markers have appeared for all EU countries using different combinations of dyes and markers. There are suggestions for electrochemical [23, 24], spectrophotometric [22, 25], gas chromatographic [26, 27] and HPLC [28] analytical methods to determine chosen fuel dyes or markers. But the variety of fuel dyes and markers set up the problem if country

has not adopted a method for their own dyes and markers because there is no suitable analytical method to determine all fuel dyes or markers in one run.

The aim of the doctoral thesis consists of two parts. Aim of the first part was to develop the column for gas chromatography with shell from an electrically conductive material which allows us lately to exploit column shell not only as a frame, but as well as a heater for direct heating of stationary phase and the volume inside it. Then to test this column using compounds with different properties for suitability for future experiments.

The aim of the second part was to develop and validate methodology for fuel dyes analysis using high performance liquid chromatography (HPLC).

The results obtained on the specific evaluation of an in-house prepared stainless steel capillary column coated with a PDMS stationary phase using demanding test probes and development of HPLC method for the analysis of dyed diesel fuel samples shows novelty and originality of this PhD thesis. For this reason, the tasks to achieve the main goals were formulated as follows:

To develop an in-house made stainless steel capillary column with a methyl polysiloxane stationary phase.

To investigate and evaluate the column properties performing demanding test probes and to compare with ones of the commercially available column.

To develop and optimize a HPLC methodological approach for the determination of fuel dyes and markers.

To adopt the developed HPLC method for sensitive detection and accurate determination of the concentrations of the dyes and the marker in fuels (SR 19 and SB 35; and the marker: SY 124) using single injection.

2. OBSERVATION OF LITERATURE

2.1. Gas chromatography (GC)

Gas chromatography is technique of separation of materials where gases act as a carrier for some mixes of volatile materials passing through the zone containing sorbent able to absorb components of these mixes. Moving speed of these materials through the sorption zone depending on intensity of interaction of these materials and sorbent. Since material interaction strength with sorbent varies depending on materials molecules size, geometry, functional groups there is always be some differences in it. These differences finally will be seen as different material moving through the sorbent area speed.

2.1.1. Historical overview and brief fundamentals

Gas chromatograph as an equipment should has: a) carrier gas handling system; b) sample introduction part; c) sample separation compartment and d) separated sample components registration part. Through the long way of development of gas chromatography technique there were made a lot of innovations on each mentioned part, but despite any updates only upper mentioned parts containing apparatus could be called gas chromatograph.

According to various sources [29-31] beginners of gas chromatography might be considered the group of scientists of the University of Innsbruck, Austria leaded by Erika Cremer and British scientists A.J.P. Martin and A.T. James from National Institute for Medical Research, Mill Hill, London. Both these groups made their research in 5th decade of 20th century. British scientists in their work described desorption of volatile fatty acids from sorbent by ethyl acetate vapors [32] as an application work. Whilst Erika Cremer and her group in their works looked to phenomena of elution different materials through tubes loaded with sorbents by gases as to separate technique and they were the ones who develop the apparatus which should be called first prototype of gas chromatograph [29, 33] which was used in Cremer's student F. Prior PhD thesis work. This apparatus, listed in Figure 1, has all components necessary for nowadays modern gas chromatograph. Additionally should be said that these two groups were focused on different approaches. British scientists investigated how to separate volatile component mixes using liquid sorbent and by later authors are recognized as pioneers of liquid-gas separation technique. Austrian group investigated gas sorption on sorbent energies and their work were recognized as solid-gas separation

technique. The connecting component of these two groups was usage of gas as a carrier. Also should be mentioned that the Austrian group in their early work used a novel detection device which is prototype of currently very wide used thermoconductivity detector [33].

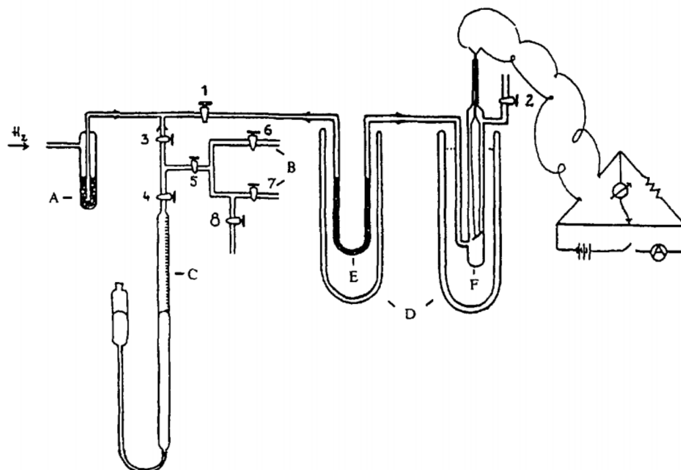


Figure 1. The apparatus used in Cramer's and Prior's work A- Adsorbent for purification of the carrier gas (H_2); B- Sample inlet system; C- burette containing mercury with niveau glass for sample introduction; D- Dewar flasks; E- Separation column (1 cm O.D. containing in 20 cm length silica gel or activated charcoal); F- thermal conductivity detector; 1-8 are glass stopcocks. A vacuum pump was connected to the system at stopcock 8.[29]

At that time there were huge demand of separation of organic compounds in oil industry. Immediately after publication of Martin and James several companies in Great Britain and The Netherlands have started their own research. Thus, additional acceleration in development gas chromatography, both instrumentation and separation theory was achieved [34]. Very soon separation units have been shaped in coiled cylinders with diameter 1-2 cm and length 2 m and more. These columns have been prototypes of nowadays still available packed columns. The ability to change column temperature during separation process has been also invented [35].

Another huge improvement in gas chromatography instrumentation were invention ionization detectors in 1958. Two types of ionization detectors were present almost simultaneously [36]. Argon ionization detector invented by J. E. Lovelock [37]. This detector has two electrodes and were filled with argon or other noble gas. Applying high voltage between electrodes created some discharge current in reaction cell. All organic compounds have lower ionization

potentials than noble gases and when such substance comes to reaction cell it ionized more easily and changes in discharge current could be fixed. In next two years author made additional improvements on the argon ionization detector and it transformed to today very well-known electron capture detector (ECD) [38, 39]. Main improvement was to use as an ionization source radioactive material. In the author's reported work they use radium isotope. Novadays ECD detectors use safer and cheaper Ni63 isotope. These type of detectors have particular role in environmental protection application. Prototype of flame ionization detector were offered by two independent groups I.G. McWilliam and R.A. Dewar in the Central Research Laboratories of Imperial Chemical Industries of Australia and New Zealand (ICIANZ) [40] and J. Harley, W. Nel and V. Pretorius from Department of Physical Chemistry, University of Pretoria in South Africa [41]. They observed that the resistance of hydrogen flame in air strongly depends from availability of vapors of organic material in the flame. Flame ionization detector has very simple construction what allows it construct very small comparing with previously available detectors it also has been very stable, has high linearity and most important it appears at the same time when an open tubular capillary columns.

From the beginning of the gas chromatography instrumentation there was not paid particular interest in separation container - column. There was not found written data due to specific requirements for early column dimensions. Since in early gas chromatography researchers built their own apparatus most likely size and shape of the columns were chosen according to its fitting to theirs apparatus possibilities. In generally columns have quarter inch (6.35 mm) width, have been filled with solid particles, in case of liquid-gas chromatography, solid particles were covered with liquid sorbent layer [42].

There in parallel with gas chromatography instrumentation development was very active development of separation theory [43-45]. In 1955 Swiss origin American scientist Marcel J. E. Golay published work with mathematical model of separation process in column [46]. Experimental data has some discrepancies form the theory. Deviation in experimental data from theory Golay assign to column geometry. Creating a mathematical model, Golay envisioned channels between the column fillers. In order to simplify the theoretical model he constructed in his mind a model, consisting of a pack of capillaries, each of these corresponding to a passage through the column packing. These theoretical capillaries would not be restricted by the geometry of the packing or by the randomness of the passages through it, which are beyond of control; therefore, they should behave close to the theoretical possibilities [47]. His complete theory of open tubular columns were presented in the international Gas Chromatography symposium in May of

1958 in Amsterdam [48]. This paper is considered as a starting point of open tubular column epoch in gas chromatography [42; 47; 49].

Every process involved surface and any media moving over it might be considered as a case of chromatographic process. In order to describe this process qualitatively, it is necessary to identify each component of the system and isolate the whole process. In terms of chromatography, surface could be called stationary phase, media moving over the surface – mobile phase. Any substance able to be dissolved in moving media – mobile phase exhibits chromatographic elution process in system stationary phase – moving mobile phase. This substance or solute, taking place in the elution process, every moment exhibits some level of equilibration being adsorbed on stationary phase (or absorbed if stationary phase is liquid) and dissolved in mobile phase. This equilibration in chromatography is called distribution constant:

$$K_c = \frac{[A]_S}{[A]_M} \tag{1}$$

Where: K_c- distribution constant; $[A]_S$- concentration of solute A adsorbed on stationary phase; $[A]_M$- concentration of solute A dissolved in mobile phase.

As we could assume, beside material dependent parameters, value of solute distribution in system phases depends from stationary phase thickness (ability to accommodate solute) or volume and volume of mobile phase above the stationary phase. Having in mind that most common form of chromatography column is cylinder, we could calculate these volumes

$$V_s = 2\pi R_c d_f h \tag{2}$$

$$V_M = \pi (R_c - d_f)^2 h \tag{3}$$

Where R_c- column diameter; d_f- thickness of stationary phase; h- length of cylinder or equilibration zone. Ratio of these volumes, or phase ratio could be expressed:

$$\beta = \frac{V_M}{V_S} = \frac{\pi (R_c - d_f)^2 h}{2\pi R_c d_f h} = \frac{(R_c - d_f)^2}{2 R_c d_f} \tag{4}$$

In capillary columns stationary phase thickness varies from 0.1 up to few µm, while column radius in practice varies from 50 to 260 µm. So we could safely assume that:

$$R_c - d_f \sim \approxeq R_c \tag{5}$$

The equation (4) with assumption in equation (5) could be rewritten:

$$\beta = \frac{R_c}{2 d_f} \tag{6}$$

Equation (6) valid only for capillary (open tubular) columns.

If we divide distribution constant K_c by phase ratio β we will get parameter

$$k = \frac{K_c}{\beta} \tag{7}$$

called retention factor k. Combining formulas (4) and (7) we will get:

$$k = \frac{K_c V_S}{V_M} \tag{8}$$

The equilibrium which exhibit solute in stationary and mobile phase system is affected by moving speed of mobile phase. As shown in Figure 2 (b) movement of mobile phase disturbs equilibrium of solute in mobile – stationary phases system.

Downward mobile phase stream amount of solute in mobile phase become enricher than in equilibrium and upward of mobile phase stream amount of solute in mobile phase become impoverished than in equilibrium. Because of these deviations solute from mobile phase front migrates to stationary phase and vice versa solute being in trailing part in stationary phase migrates to mobile phase. Such cyclical process is simple model how the solute moves through the column. Also should be taking into account that certain volume of stationary phase is able to absorb/adsorb only certain amount of solute. In ideal case, when stationary phase absorption capacity is much higher than amount of the solute absorbed being in equilibrium in mobile-stationary phase system, distribution of the solute takes Gaussian distribution shape (Figure 3).

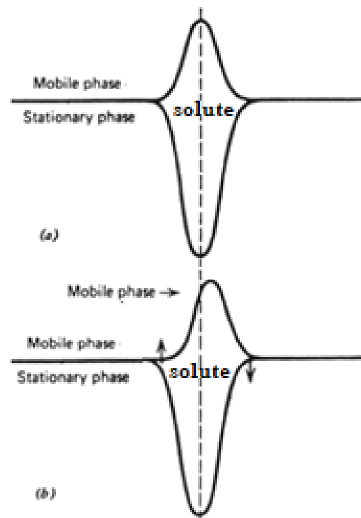


Figure 2. Solute distribution in system mobile phase – stationary phase. (a) – solute are in equilibrium in system mobile phase – stationary phase; (b) – solute equilibrium is disturbed by the movement of the mobile phase.[50]

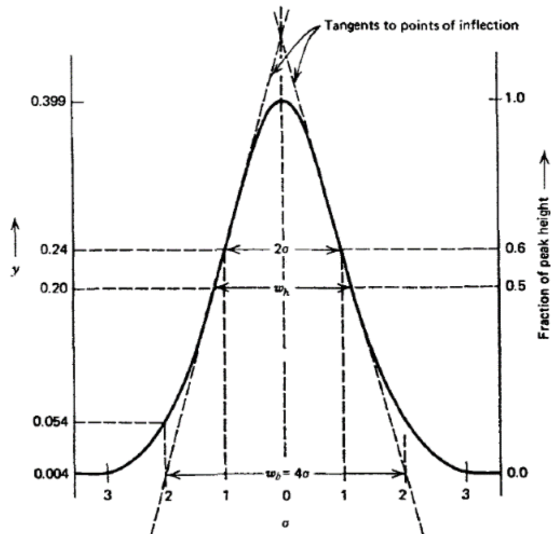


Figure 3. Normal distribution of the solute [52].

It is considered that in gas chromatography mobile phase does not interact with stationary phase or this interaction is negligible. Time which is necessary to mobile phase to go through the column is called mobile phase hold-up time t_M. Meanwhile, the elution time of the solute t_R consists of its presence in the mobile phase, which is equal to the elution time of the mobile phase t_M and its presence in the stationary phase $t_R^{'}$ which is the rest part of the elution time t_R (see Figure 4).

$$t_r = t_M + t_r' \quad (9)$$

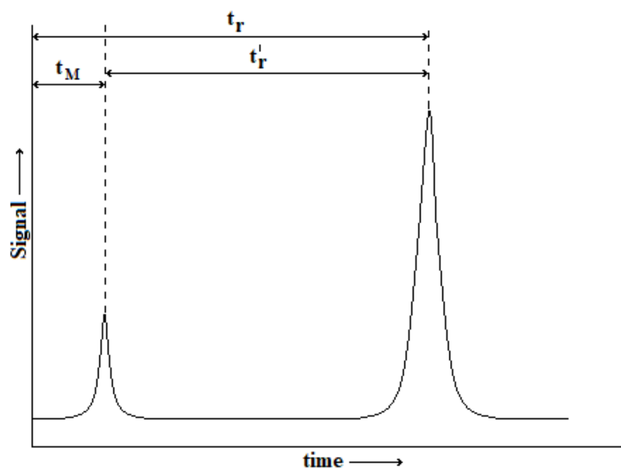


Figure 4. Solute elution. t_R – solute elution time; t_M – mobile phase hold-up time; $t_R^{'}$ – solute elution time

Time wich is necessary for mobile phase to pass through the colum is proportional for the mobile phase volume V_M needed to fill column, on the other hand time necessary to elute solute is proportional to mobile phase volume V_r. Then we could rewrite Equation (9):

$$V_r = V_M + V_r' \quad (10)$$

Ratio of t_r' or V_r' and t_M or V_M is dynamic expression of value of retention factor k

$$k = \frac{t_r'}{t_M} = \frac{V_r'}{V_M} \quad (11)$$

Combining equations (8) and (11) and their expression V_r', inserting into equation (10) we get the following expression:

$$V_r = V_M + K_c V_S \quad (12)$$

From equation (12) is it seen that total volume of mobile phase V required to eluate a solute consist from two parts: amount of V which is represent column volume and which solute should pass through while it is in mobile phase and volume of mobile phase $K_c V_S$ which is pass through while solute is absorbed in stationary phase.

By knowing column length L and mobile phase hold up time it is possible calculate mobile phase linear velocity $\bar{u}$:

$$\bar{u} = \frac{L}{t_M} \quad (13)$$

As well knowing column length L and solute elution time it is possible calculate solute average velocity μ through the column:

$$\mu = \frac{L}{t_R} \quad (14)$$

Ratio of solute average velocity μ through the column and mobile phase liner velocity $\bar{u}$ is called retardation factor:

$$R = \frac{\mu}{\bar{u}} \quad (15)$$

Retardation factor value is always less than 1 and represent ratio at which solute moves respectively to mobile phase through the column. Combined equations (13), (14) and (15) gives:

$$R = \frac{t_M}{t_R} = \frac{V_M}{V_R} \quad (16)$$

Inserting equation (12) to equation (16) gives:

$$R = \frac{V_M}{V_M + K_c V_S} \quad (17)$$

or with help of equations (8), (10) and (11) get relation between two chromatographic constants R and k:

$$R = \frac{1}{1 + k} \quad (18)$$

The essence of chromatography is to separate the different components, so it means that there are more than one solute in chromatographic process. If separation of chromatographic system is not enough for certain pair of solutes, these solutes are eluted simultaneously. Such elution of solutes are called co-elution. Qualitative characteristic of separation of solutes in chromatographic system are expressed by separation factor α.

$$\alpha = \frac{t'_{r(1)}}{t'_{r(2)}} = \frac{k_1}{k_2} = \frac{K_{c1}}{K_{c2}} \quad (19)$$

Higher separation factor values indicates better stationary phase suitability for certain pair of solutes. In other words the higher separation factor represents better stationary phase selectivity for respective pair of solutes. Separation factor holds inside information about difference of solutes moving speed through the column and useful than choosing stationary phase, but it says nothing about the shape of the solute.

The hypothetical pair of solutes is illustrated in Figure 5.

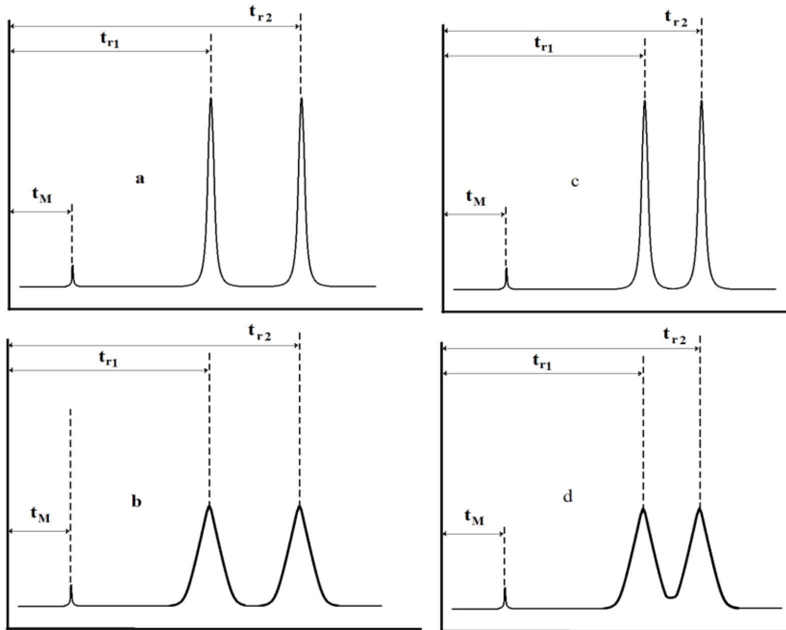


Figure 5. Separation factor and solute band broadening.

In parts a and b solutes have same retention times or their separation factor is same, but there is different interaction behavior of solute in column. In part a solutes shapes or peaks are higher and narrower than in part b. In parts c and d peak shapes are same as in parts a and b but retention time differs. If in part c peaks are still resolved whereas in part d some overlapping are visible. Peak broadening depends from column size (volume of open area in column), solute and stationary phase nature, stationary phase thickness, mobile phase velocity and diffusion of solute molecules.

Defining resolution is very important to characterize the peak shape. In normal chromatography cases peak shape corresponds to normal or Gaussian distribution [50]. The peak of a solute represents all the molecules of the solute moving through the column as a unit. According to statistics in normal distribution case [51, 52] 68 % of values fall in 1 standard deviation of mean or 2σ and 95 % in 2 standard deviation of mean or 4σ. Figure 3 represents normal distribution form and σ represents standard deviation. 2σ in normal distribution is at 60 % of peak height. Due to simplicity describing peak shape is used peak width at half height w_h witch represent of 2.354 σ, or at the peak base w_b at 4σ. Based on mentioned above and as shown in Figure 6 resolution R_S could be described as:

$$R_S = \frac{(t_r)_B - (t_r)_A}{\frac{(w_b)_A + (w_b)_B}{2}} = \frac{2d}{(w_b)_A + (w_b)_B} \tag{20}$$

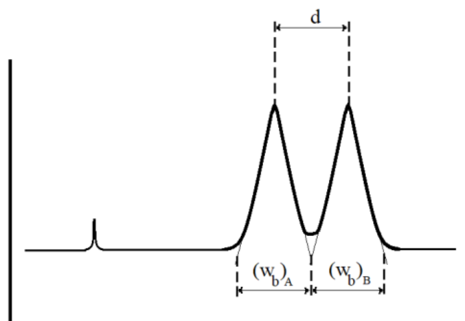


Figure 6. Peak resolution illustration.

Where d represent time between peak maxima, $(w_b)_A$ and $(w_b)_B$ peaks widths at base.

Another widely used characteristic for evaluate column efficiency is plate number N. It is comes from analogy with distillation process:

$$N = \left(\frac{t_r}{\sigma}\right)^2 \qquad (21)$$

For normal distribution peaks σ could be expressed as peak width. If use peak width at base w_b witch corresponds 4σ:

$$N = \left(\frac{t_r}{\frac{w_b}{4}}\right)^2 = \left(\frac{4t_r}{w_b}\right)^2 = 16\left(\frac{t_r}{w_b}\right)^2 \qquad (22)$$

Similarly for peak width at half height which corresponds to 2.354σ:

$$N = \left(\frac{t_r}{\frac{w_h}{2.35}}\right)^2 = \left(\frac{2.35t_r}{w_h}\right)^2 = 5.54\left(\frac{t_r}{w_h}\right)^2 \qquad (23)$$

The plate number indicates how many equilibrations occurs while solute are eluted. [53-55]. To make useful comparisons between different lengths columns is used another parameter – plate height H:

$$H = \frac{L}{N} \qquad (24)$$

2.1.2. Modern applications

The areas of application of gas chromatography are very wide, consequently it is impossible to cover all of them in very short survey. Therefore, only selected topics will be observed in this part of PhD thesis. For instance, in 2001 the possibility of applying headspace microextraction into a single drop for the determination of alcohols in aqueous solutions using gas chromatography was successfully demonstrated [56]. It was the beginning of fast development and adoption of greener and sustainable microextraction techniques as an effective alternative to classical sample preparation procedures. The headspace analysis, one of the most popular methods of sample preparation for determining, first of all, volatile organic substances in liquid and solid samples. The possibility of applying headspace microextraction for the determination of different analytes are summarized in several reviews [57-60].

Sample preconcentration is one of the most time and labour consuming steps of the analysis and though the development of fast and simple extraction techniques is always of interest. It was noticed that upon water solutions a hydrophilic ethylene glycol drop increased significantly and at higher sampling times grew so much that it even lost the contact with the needle. In order to overcome this problem, instead of hydrophilic ethylene glycol, hydrophobic o-xylene was suggested [61]. The role of some important factors such as sampling

temperature and time, stirring rate, and ionic strength of the solution on the extraction efficiency was determined in this study. The proposed method is simple, cost-effective and allow to use very small amount of toxic organic solvents. This enabled the applicability of the headspace single drop microextraction for the analysis of different analytes to date [62-64].

The applicability of headspace and direct microextractions to a single drop, in order to determine several esters, has been also studied. A drop of p-cymene containing n-nonane as an internal standard was used for extraction [65-68]. The characterization of aromatic profiles of different food products was also performed. These results allow to understand better the aromatic compounds in different fruits and to determine the characteristic aromatic components.

A simple, rapid and inexpensive procedure for extraction of volatile halogenated hydrocarbons by headspace single drop microextraction was presented in [69]. Decane was selected as the extracting solvent. The operation parameters such as organic drop volume, sample stirring rate, extraction time and salt concentration were optimized. The optimum extraction conditions were the following: 0.8 g of NaCl was added to the extraction vial containing 2 ml of extraction solution. Sampling was carried out into a 2 μl decane drop for 20 min at a 150 rpm stirring rate. A chromatogram of the standard solution of halogenated hydrocarbons, obtained after HS-SDME in optimized extraction conditions, is presented in Fig. 7.

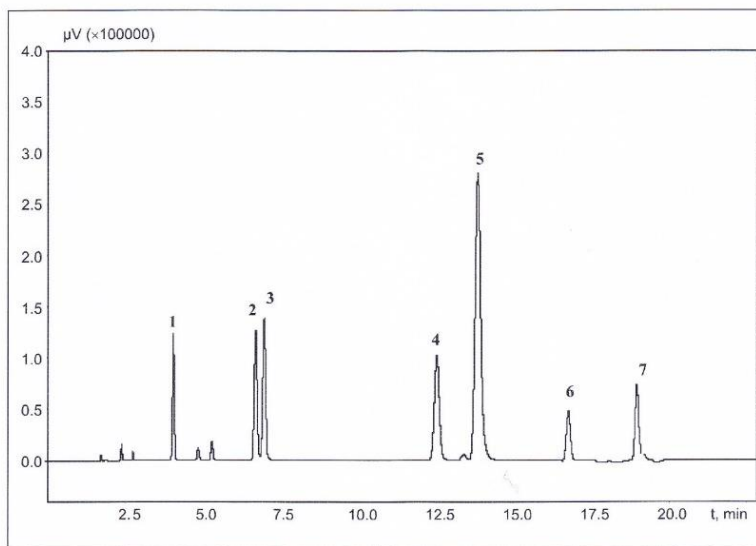


Figure 7. Chromatogram of standard solutions of (1) chloroform, (2) trichloroethene, (3) bromodichloromethane, (4) dibromochloromethane, (5) tetrachloroethene, (6) chlorobenzene and (7) bromoform, obtained after HS-SDME in optimum conditions. The concentrations of the analytes are 10 mg/l.

The repeatability of the method was 5.0–6.5%. Calibration graphs were linear up to 50 µg l–1 ($R^2 \geq 0.995$) and detection limits ranged from 0.42 to 1.78 µg/l. For all the target compounds, the limits of detection were much lower than the maximum contaminant level permitted in drinking water. The proposed technique was applied for volatile halogenated hydrocarbon determination in groundwater (see Fig. 8).

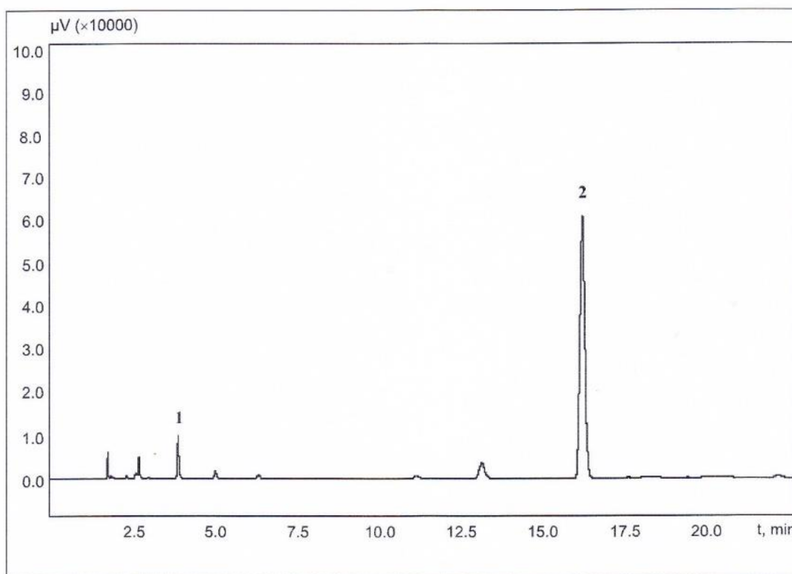


Figure 8. Chromatogram of groundwater obtained after HS-SDME in optimum conditions: (1) chloroform, (2) chlorobenzene.

Static headspace-gas chromatographic analysis was suggested for acetaldehyde, ethanol, acetone, and acetoin determination in yogurts: "Turkisk", "Aistė Natural with Honey", "Dobilas Natural" and "Dobilas with Lemon and Ginger" [70]. The quantification was made using the standard addition method. Yogurt was diluted with distilled water at the ratio 1:1. Three portions of the mixture (12 g each) were analysed: the first portion was not spiked, the second portion was spiked with 100 µl of the standard solution containing 100 mg/l of each analyte, and the third portion was spiked with 200 µl of the standard solution. The results of the analysis were in correlation with those presented in the literature: 2–80 mg/kg for acetaldehyde, 1.8–11 mg/kg for acetone, 0.2–45 mg/kg for ethanol and 2.2–28.2 mg/kg for acetoin. The only exception was determined concentration of ethanol in "Dobilas with Lemon and Ginger". This yogurt is flavoured and likely the peak of added flavour interferes with that of ethanol. Thus for the analysis of this yogurt type, gas chromatographic conditions should be modified in order to achieve better separation of volatile components.

The headspace gas chromatographic analysis was suggested by the same scientific group for the determination of acetic and propionic acids in aqueous media [71], and for the determination of lactic, oxalic, succinic, malic and citric acids [72].

In the last decade, the development and adoption of greener and sustainable microextraction techniques have been proved to be an effective alternative to classical sample preparation procedures. It is known about 10 commercially available solid-phase microextraction systems with special attention to the appraisal of their analytical, bioanalytical, and environmental engineering [73;74].

In many review articles an overview of the challenges and achievements in the application of fully automated miniaturized sample preparation methods in analytical laboratories are provided. Both theoretical and practical aspects of these environment-friendly preparation approaches also have been discussed. For example, a solid-phase microextraction fibre based on the electrochemically deposited polyaniline– polypyrrole coating has been used for the extraction and consequent gas chromatographic determination of parabens [75]. The repeatability of the method was determined by five repetition analysis of two different concentrations of the analytes. The limits of detection were found to be much lower for ethylparaben and propylparaben likely due to their lower solubility in water in respect of methylparaben.
A new solid phase microextraction (SPME) fibre using carbon nanotubes as fibre coating incorporated into a groove of a stainless steel rod has been suggested by Adomaviciute et al. [76]. The SPME device was modified from a commercial SPME device [a SPME fibre holder for manual use (Supelco, St Louis, MO, USA)]. A stainless steel plunger needle was used as a support for a coating. The plunger needle was mounted inside the external needle, cleaned with acetone in an ultrasonic bath and dried at room temperature. A thin layer of epoxy glue was spread on the groove surface of the plunger needle and a CNT powder was gently pressed to the glue. The schematic view of the device is presented in Fig. 9. The surface of the coating was examined by scanning electron microscopy (SEM). A groove of the plunger needle coated with CNT powder is evidenced in the SEM micrograph (Fig. 10). The results obtained in this study showed that SPME using stainless steel support coated with CNT powder offers an attractive alternative to commercially available fibres. The fibre was mechanically stable, exhibited relatively good repeatability and high thermal stability. As the fibre coating was incorporated into a groove of a stainless steel rod, the sorbent particles did not contact the outer needle of the SPME device and thus the mechanical damage of the coating was prevented. The coating selectively extracted aromatic hydrocarbons in the presence of

polar compounds. Moreover, the sorbent layer was rather thick resulting in high extraction efficiency of highly volatile analytes. However, in order to avoid the contamination of the fibre with low volatility compounds, headspace SPME should be applied for the determination of volatile analytes in complex matrices.

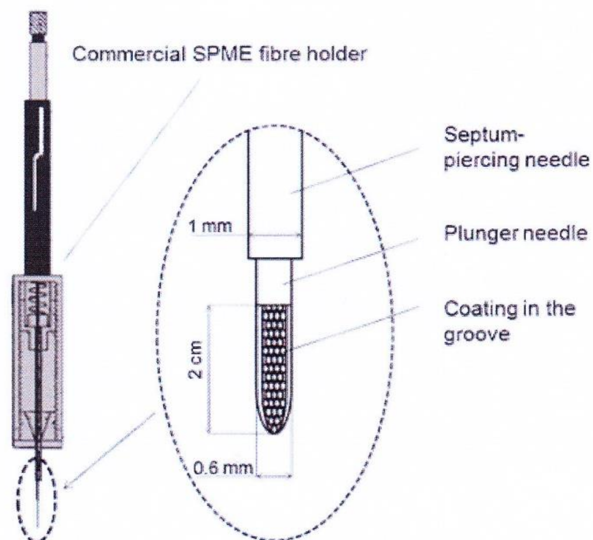


Figure 9. A schematic diagram of the modified SPME device. [76]

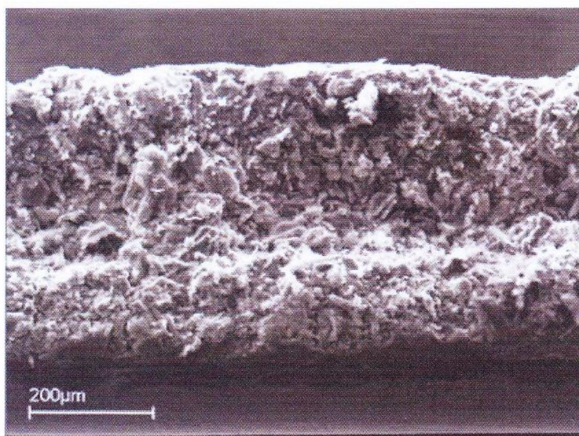


Figure 10. SEM micrograph of SPME fibre coated with carbon nanotubes [76].

We are convinced that green analytical chemistry will be really useful in the years ahead by many recent publications. The application of cheap, fast, automated, "clever", and environmentally safe gas chromatographic procedures to environmental, clinical, and food analysis will improve significantly the quality of the analytical data.

2.2. High performance liquid chromatography (HPLC)

Liquid chromatography is technique of separation of materials where some liquid act as a carrier for some mixes of materials soluble in this liquid and passing through the zone containing sorbent able to absorb components of these mixes. In terms of chromatography carrier are called mobile phase, a mix of material eluted by mobile phase is called solutes, sample or analytes, an absorption zone is called stationary phase. The essential difference between gas and liquid chromatography is the state of mobile phase. While in gas chromatography mobile phase is in gaseous state, in liquid chromatography mobile phase is liquid.

2.2.1. Fundamentals

Since liquid is a denser medium than gas, the migration of sample components through the mobile liquid phase toward stationary phase is slower, it requires to use stationary phase particles as small as possible to reduce the gaps between particles and migration time. In addition, liquids are more viscous media than gases, and reducing space between particles requires power to displace these liquids through the stationary phase particles. These two opposite effects requires some balance between power or pressure applied to the liquid chromatography column and stationary phase particle size [77]. Over time, the particle size of the sorbent used was found to be 3-5 µm. In order to pass the liquid through a chromatographic column 10 to 25 cm long loaded with particles of this size, a pressure of 50 to 500 bar is required, depending on the viscosity of the liquid used. Because of so high pressure and requirement to balance between choice of particle size and pressure, term of Liquid Chromatography changed to High Pressure or High Performance Liquid Chromatography - HPLC. In most recent years, machines able to supply steady in around 1300 bar pressure were developed [78-80]. It allows to reduce stationary phase particles size up to 1,7 µm. These techniques are called ultra-high performance (pressure) liquid chromatography – UHPLC.

Unlike in gas chromatography where sample components interact only with stationary phase, in liquid chromatography eluated materials interact

with both mobile and stationary phases. Also, mobile phase by itself interacts with stationary phase and this interaction competes with sample – stationary phase interaction.

If the suitability of a material for usage in gas chromatography is determined by its volatility. The boiling point of the material must be less than 500°C and the vapour pressure of the material in separation conditions should be in range at least few kilopascals. The suitability of a substance for usage in liquid chromatography is determined by its solubility in the mobile phase, which would be analogous to volatility in gas chromatography. Unlike the volatility of the sample material in the case of gas chromatography, the solubility of the sample material depends not only from the material itself but also from the solvent or, in the case of liquid chromatography, from the properties of the mobile phase. A sample material dissolved in a solvent can dissociate into ions, which also affects the behaviour of the sample material in chromatography system. In some cases, dissociation is undesirable and is intended to be avoided by modifying the mobile phase so that dissociation of the sample molecules does not occur. In other cases, the essence of the chromatographic separation consists in the interaction of the dissociated particles with the stationary phase and the mobile phase is selected so that the molecules of the sample are completely dissociated.

In addition to the behaviour of the sample molecules in the mobile phase, it is very important that the interaction of the sample molecules with the stationary phase is stronger than that of the mobile phase with the stationary phase. However, this interaction must not be too strong and the value of the sample distribution in the stationary and mobile phases K_c (1) should not be neither very high nor very small. So it should be taking into account when selecting stationary phase for certain chromatographic application. Sample components whose K_c value is lower or k value (11) close to 1 is eluting faster than these whose values are higher. Stationary phase might be solid or liquid immobilized on solid particles and from it depends interaction nature of sample components with stationary phase. Main types of interaction could be listed as follow:

1. Partition interaction. It occurs then molecules of sample are distributed between two liquid phases. One of the liquid phases is immobilized on the solid particles surfaces and act as a stationary phase, another liquid phase is mobile phase. Liquid immobilisation could be achieved when the molecules of very polar liquid makes hydrogen bonds between themselves and surface of solid particle and when non polar mobile phase is rinsing these particles. Partition interaction are illustrated in Figure 11.

As an example of such immobilized liquid could be used water adsorption on the surface of silica oxide. Another liquid immobilisation could be achieved by bonding long chain organic molecules layer to the solid particles surfaces. Polar solvent should be used in this case. Various species of molecules having different level of polarity exhibits different level of distribution between both of these liquid phases and moves through the system in accordance with their distribution level.

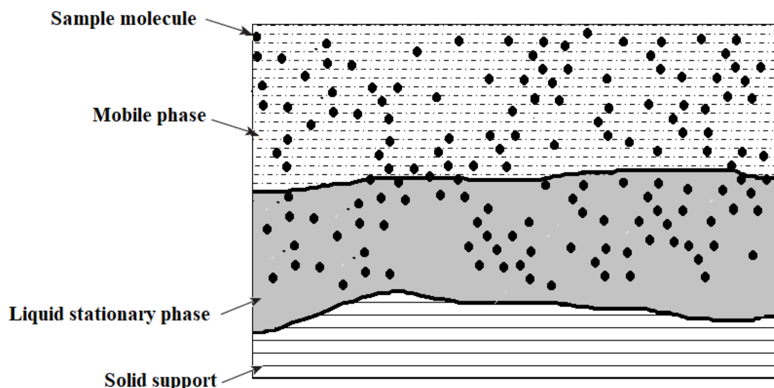


Figure 11. Partition interaction.

2. Adsorption interaction. This type of interaction occurs between solid and liquid phases. Sample molecules distributes between liquid mobile phase and solid stationary phase surface. Similarly as in partition distribution, if stationary phase are more polar than mobile phase, more polar molecules spent more time adsorbed on surface and eluate later than less polar molecules. Schematic example of adsorption interaction are shown in Figure 12.

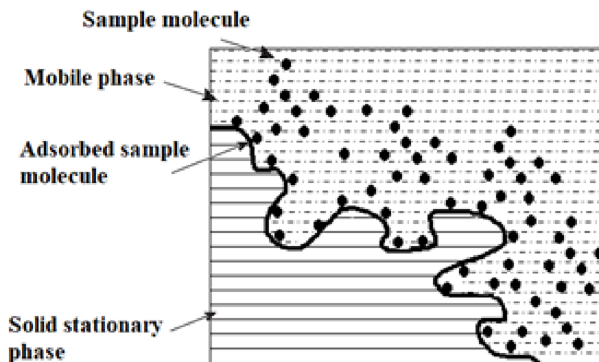


Figure 12. Adsorption interaction.

3. Ionic interaction. This type interaction occurs than sample molecules in mobile phase are dissociated into ions and stationary phase contains functional groups whose are able attract ionic species from mobile phase. Schematic example of ionic interaction are shown in Figure 13.

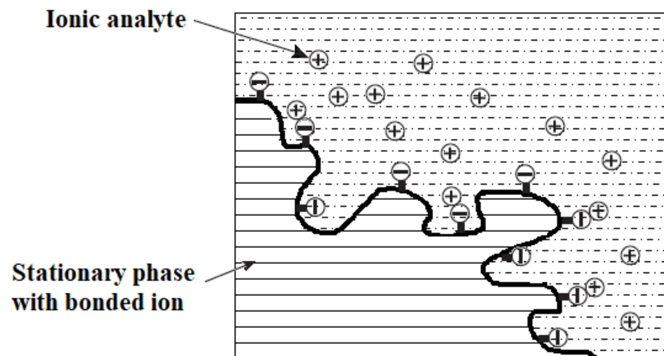


Figure 13. Ion exchange interaction.

Interaction based on size of sample molecules. In this type of interaction stationary phase are made from porous structure. Small sample molecules from the mobile phase could to enter into pores while large molecules couldn't. Finally, larger molecules pass through the stationary phase structure faster than smaller molecules whose velocity of movement is limited by their ability to enter the internal cavities of the porous structure. Schematic example of interaction based on size of sample molecules are shown in Figure 14.

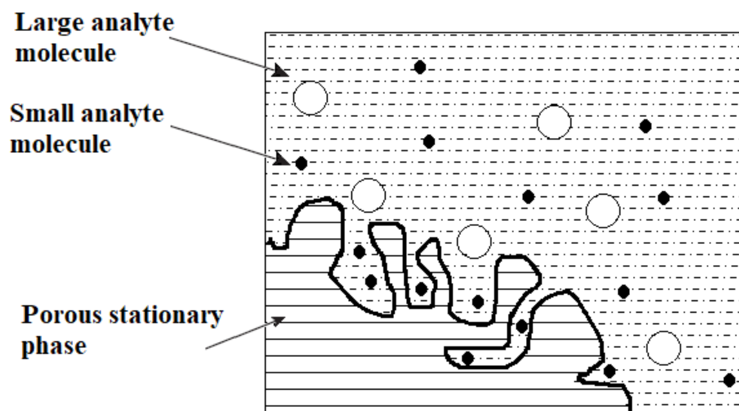


Figure 14. Interaction based on size of sample molecules

4. Affinity interaction. This is very specific interaction type where the stationary phase has sites in a structure. This is a very specific interaction in which there are places in the stationary phase structure that correspond to the geometric profile of the sample molecule or its part. This interaction is widely used in the separation of biologically active substances.

First two interaction types mentioned above take place in both in liquid and gas chromatography, while ionic and interaction based on molecule size is unique for liquid chromatography. Another unique feature of liquid chromatography is the ability to change the composition of the mobile phase during the chromatographic process. Processes where mobile phase composition is same during whole process are called isocratic chromatography, when mobile phase composition changes during process are called gradient chromatography. Depending on the type of interaction there could be used a gradient mode of polarity, pH or ionic force.

Combination of these interactions makes liquid chromatography much more complex technique than gas chromatography. On the other hand complexity gives much more possibilities. The versatility of the interactions allows the selection of very specific conditions under which very delicate mixtures of analytes can be separated. Also such a variety of interactions requires some classifications to be easier handle the types of liquid chromatography. There is listed main types of liquid chromatography [81] and short description of the principle of the separation:

1. Reversed phase liquid chromatography or RP-HPLC. This is most common type of liquid chromatography. There polar mobile phase and nonpolar stationary phase are used. Very large number of compounds could be separated by this type of liquid chromatography. The basic requirement for the molecules of the substance is that they would have a hydrophobic and hydrophilic moiety. Most common stationary phase used in this type of chromatography is immobilised liquid. Main of them is C18 hydrocarbon chain bonded to silica oxide or other natural or artificial particle surface. Depending on application this chain could be modified by functional groups like $–NH_2$ or –CN. As a mobile phase most commonly are methanol and acetonitrile. These are selected because of their low light absorbance in ultraviolet (UV) light range. In case of gradient process or when more polar than solvent is necessary some ultrapure water might be added. If necessary to stabilize pH or ionic force small amount of acid, base or buffer could be added. Sample molecules having large nonpolar moiety moves to nonpolar stationary phase more easily and stay there longer than molecules with smaller nonpolar moiety. Their retention in column are longer.

2. Hydrophobic interaction chromatography (IPC). This type of chromatography is similar to RP-HPLC, except that there an ion-pairing reagent to mobile phase are added. Ion-pairing reagent have a large ionic molecules with charge opposite to the sample component molecules, also substantial hydrophobic region that allows interaction with the stationary phase [82; 83].
3. Hydrophobic interaction chromatography (HIC). This type of chromatography is mainly used in proteins and other biomolecules separation. There large protein or biopolymer molecules, containing both hydrophilic and hydrophobic parts are eluted through HIC column under high salt buffer conditions. Hydrophobic effect of mobile phase could be increased by adding salt, usually ammonium sulphate. It leads to easier biopolymer adsorption on the stationary phase [84]. Reversely, decreasing salt amount in mobile phase leads to polymer desorption. Varying amount of salt in mobile phase allows bind proteins from aqueous solutions in varying degrees depending on the structure of targeted protein, pH, temperature and organic solvents used.
4. Nonaqueous reversed-phase chromatography (NARP). It is type of reverse phase chromatography where is used nonpolar stationary phase and slightly polar nonaqueous solvent, usually mixture of less and more polar organic solvents capable to dissolve compounds of interest. This type of liquid chromatography is used to separate very nonpolar compounds hydrophobic substances such as hydrocarbons and glyceride oils [85].
5. Hydrophilic interaction liquid chromatography (HILIC). In this type of chromatography is still used polar mobile phase as in reverse phase liquid chromatography, usually acetonitrile with some amount of water. However stationary phase is also polar as in normal phase chromatography usually made of pure silica oxide or silica modified with amino, amide, cyano, diol, zwitterionic, cyclodextrin, saccharide or other polar functional groups. This type of liquid chromatography is as an alternative to normal phase chromatography and used to separate slightly polar acidic and basic compounds. Hydrophilic interaction chromatography have some advantages comparing to normal-phase chromatography. Since there is used mobile phase same as in reverse phase liquid chromatography, it is makes more easily to switch equipment from one application to another comparing to normal phase chromatography, as well easier adapt operational parameters to sophisticated mass spectrometer detectors [86; 87]
6. Normal-phase chromatography (NPC). This type of liquid chromatography uses polar stationary phase and nonpolar solvent usually hexane or

tetrahydrofuran. Main feature of normal phase chromatography is noncompatibility with aquatic media. Usually it is used to separate polar compounds, there less polar compounds are eluted earlier than more polar.

7. Cation-exchange chromatography. This type of liquid chromatography is used for the separation of cations both organic and inorganic. Retention of cations are based on the attraction between cations in a solution and the opposite charged of the stationary phase.
8. Anion-exchange chromatography. This type of liquid chromatography is used for the separation of anions both organic and inorganic. Retention of anions are based on the attraction between cations in a solution and the opposite charged of the stationary phase.
9. Ion-exchange on amphoteric or zwitterionic phases. This is a type of ion exchange chromatography similar to the cation-exchange or anion-exchange chromatography. The stationary phase of this type of chromatography contains functional groups that have an amphoteric character or, in the case of zwitterionic phases, both anionic and cationic groups. The mobile phase in these types of chromatography consists of buffer solutions.
10. Ion-exclusion chromatography. It is type of liquid chromatography where an ion-exchange resin is used for the separation of neutral species between them and from ionic species. In this technique, ionic compounds from the solution are rejected by the selected resin and they are eluted as nonretained compounds. Nonionic or weakly ionic compounds penetrate the pores of the resin and are retained selectively as they partition between the liquid inside the resin and the mobile phase.
11. Ligand-exchange chromatography. This is type liquid chromatography where stationary phase is cation exchange resin charged with metal ions able to form coordinative bonds with sample molecules. Elution is reached by increasing strength of mobile phase, it allows to substitute sample molecules being in coordinative bond with stationary phase by mobile phase molecules. Separation is based on differentiation of interaction strength between sample molecules with bonded metal ion. Immobilized metal affinity chromatography
12. Immobilized metal affinity chromatography. This is very similar to ligand exchange chromatography, there is stationary phase contains chelating groups that can make complexes with metals. Metal ions complexed in stationary phase still able to form complex bonds with electron donor molecules. Retained molecules could be eluted by changing pH or adding some additional displacing agent.

13. Ion-moderated chromatography. This type of chromatography is similar to ligand exchange chromatography with difference that metal cations loaded on the stationary phase do not form coordinative bonds and interactions is based mainly on polarity.
14. Gel filtration chromatography (GFC). This is type of size exclusion chromatography where the sample molecules are separated based on their size. The stationary phase is porous material having various size and length pores. Mobile phase is aqueous solution. Gel filtration chromatography is applied to samples soluble in water and other polar solvents. Smaller molecules of sample passing through porous stationary phase is able to come into smaller pores and in total travel longer path than larger molecules. Larger molecules not able to enter to pores like smaller ones, travel shorter paths and are eluted earlier than smaller.
15. Gel permeation chromatography (GPC). This is another case of size exclusion chromatography. The main difference from gel filtration chromatography is mobile phase. There is used nonpolar organic solvents. This type of chromatography is used to separate hydrophobic macromolecules from smaller ones.
16. Displacement chromatography. In this type of chromatography are initially retained on stationary phase. This initial stage is called loading phase. After sample is loaded on the column, some displacement agent able to eluate specific molecules of sample is added for eluting them. There is could be used one or several stages of elution depending displacement agents selectivity to sample.
17. Affinity chromatography. This is type of chromatography where the reversible biospecific interactions between a molecular species and its ligand are used for the isolation of that molecular species from its biological environment. There is a specific ligand, having appreciable affinity to interested molecular species, attached to a solid support to form a specific stationary phase. As a mobile phase there should be used a solvent which does not destroys biological activity of molecular species of interest. Mobile phase with dissolved interested molecular species pass through that specific stationary phase and only that specific molecular species are retained. Later it should be eluted using stronger solvent without destroying its biological activity [89].
18. Chiral chromatography on chiral stationary phases. This is a type of chromatography for separation of chiral compounds also called optical isomers. It differs from the regular chromatography that there stationary phase is specially prepared for specifically retain one of the enantiomers of the specific compound of interest [90].

19. Chiral chromatography on achiral stationary phases. This type of chromatography is a separate technique of separation of chiral components. There are used some modifiers which interact only with one of the enantiomers of compound of interest. Later these the modified and nonmodified enantiomers could be easily separated by conventional liquid chromatography methods.
20. Multimode HPLC. This is a type of chromatography where used columns contains more than one type of stationary phase, for example, some with bonded nonpolar groups like C18, and some with ionic groups like SO_3-. In most cases, presence of two types of interactions like a polar and a hydrophobic is not desirable, but in some cases dual properties of a stationary phase can be used as an advantage of the separation.

Table 1. Relationship between interaction type and liquid chromatography type

Separation mechanism	Chromatography type
Hydrophobic forces	reversed phase, ion pair, hydrophobic interaction chromatography, non-aqueous reversed phase.
Difference in polarity	hydrophilic interaction liquid chromatography, normal phase,
Ion interaction	cation exchange, anion exchange, ion exchange on amphoteric and zwitterionic phases, ion exclusion, ligand exchange, immobilized metal affinity, ion moderated
Size exclusion	gel filtration, gel permeation
Displacement	displacement
Bioaffinity	bioaffinity
Chiral	chiral stationary phase, chiral mobile phase
Various principles together	multi-mode

There is not always easy to identify what type of interaction or equilibrium which exhibits sample molecules in certain type of chromatography, but it could be associated as listed in Table 1.

Up to now there were listed information about possible materials separation ways in liquid chromatography. Another very important part of liquid chromatography is detection of molecules of interested components after they are separated. Detection is based on different physicochemical properties of these molecules neither molecules of mobile phase. There are many detection principles of eluted sample. Most of them are non-destructive

and allows to be connect two or even more detection devices in series depending on the necessarily. Main types of detectors would be:

1. Ultraviolet and visible light absorption (UV-VIS) based detectors. Principle of these detectors are based different light absorption of component molecules neither mobile phase. When the sample components passing through the flow cell of detector they absorb some light and some deviation are observed on light intensity registration unit. These detectors could be constructed to collect absorption data of single light wave or wide range of light spectra and usually collect data in interval of light wave length from 190 to 700 nm. These detectors are very widely used because of their universality. The lack of these detectors is insufficient sensitivity for some classes of compounds.
2. Fluorescence detector. Principle of this type of detector based on sample substance ability to absorb photon of certain light wave and emit photon of longer wavelength. When sample component is passing through the sample cell it is illuminated by certain wavelength which are called exciting wavelength and emits some photon of longer wavelength called emission wavelength. These detectors are very sensitive for the components which are sensitive for fluorescence effect. Lack of these detectors are limited range of compounds sensitive for fluorescence effect.
3. Refractive index detector. This type of detector based on different light speed in various media. This type of detector based on different light speed in various media. The detector cell is constructed from two compartments one is reference another operational. When sample is not present in the cell, the light passing through both sides of cell is focused on the photodetector. When the sample components pass through the detector cell, the deflection of a light beam is changes. This results in a change in the photon current falling on the detector which unbalances it. Refractive index detectors are universal and sensitive for any material. Lack of these detectors is low sensitivity comparing for other detectors, as well there is not possible to use mobile phase gradient using this detector. Also this detector is sensitive to the temperature changes thus it should be used in termostating environment.
4. Chemiluminescence detector. Principle of this detector is based on that some materials reacting in some specific way can produce light. This type of detector does not have light emission source only light detection part. Sample components able to exhibit chemiluminescence after eluting from column are mixed with specific reagent which react with sample component and initiate chemiluminescence process. Reacted component flows to detector cell where emission from the material is registered. These

type of detectors are very sensitive, but there not many materials which could able to exhibit chemiluminescence.

5. Electrochemical detectors. These types of detectors are based on ability to measure electric charge transfer through the liquid phase. There are many principles how it could be realized. Among these are amperometric, coulometric, potentiometric, and conductometric techniques. Most commonly used technique in liquid chromatography is conductometric and amperometric. These detectors are relatively simple. There is flow through cell and electrodes installed in this cell. Electrical parameter are measured on the flowing mobile phase through the cell. When the sample components comes to the cell there is registered deviation of measured parameter. These detectors have very good sensitivity, but are used only in ion chromatography.
6. Light scattering detectors. These type of detectors principle is based on light interaction with nontransparent small particles. When the light beam comes through the mist of very tiny particles it is scattered. The more particles will be in the path of the light beam the more light will be scattered, also the angle by witch light is scattered depends from particle size. The mobile phase entered to detector are evaporated by inert gas flow and sample components being in mobile phase turn to solid particles. This type of detector is especially useful in conjunction of size exclusion chromatography. This type of detector is very universal also could be used with gradient mobile phase profile, but should be taken into account any salt usage in mobile phase modification, because salt particles could mask sample component response.
7. Mass spectrometry detectors. These types of detectors are most robust, most complicated, most expensive and most informative detectors. Common feature of all mass spectrometer detectors is sample component ionization and separation ionisated particles by mass-to charge ratio in electric or magnetic field. There is huge amount of sample ionization techniques such as: atmospheric pressure ionization (API); atmospheric pressure chemical ionization (APCI); electrospray ionization (ESI); matrix-assisted laser desorption ionization (MALDI); atmospheric pressure photo ionization (APPI); proton transfer ionization (PTI); selected-ion flow-tube ionization (SIFTI).

There could be separated three operational principles of mass spectrometer detectors quadrupole, time of light and magnetic field detectors. Quadrupole mass spectrometers core is four rode system where using electrical field are separated particles having different mass-to-charge ratio. Time of light devices is measuring time by which particle flies from ionization to detector.

Particles having same charge but different mass, have different acceleration in electrical field. As well particles with same mass but different charge have different acceleration too. In magnetic field detectors, a magnetic field of a certain strength acts on a trajectory in which charged particles move. Depending on the mass-to-charge ratio of the particle, the trajectory of that particle's motion changes also the time necessary for the particle to reach the detector.

Beside simple mass spectrometry technique could be applied as various combinations of mass spectrometry like a triple quadrupole (MS-MS), quadrupole – time of light (Q-TOF); time of light – time of light (TOF-TOF), in these techniques intermediate chamber is used where ionisated sample exhibits secondary ionization [91].

2.2.2. Modern applications

High-performance liquid chromatography (HPLC) is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. Each component in the analyzed sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column. During the last 20 years high-performance liquid chromatography in combination with various detection techniques has been extensively applied to the speciation of a number of metal ions. A method for on-line chromatographic preconcentration and determination of chromium(VI) traces has been developed by Padarauskas et al. [92].

A new method involving matrix solid-phase dispersion (MSPD) extraction and hydrophilic interaction ultra-high-pressure liquid chromatography (HILIC-UHPLC) with photodiode array detection was developed for the determination of carbadox and olaquindox in feed [93]. Carbadox (methyl-3-(2-quinoalinylmethylene)carbazate-*N*1,*N*4-dioxide) and olaquindox (2-(*N*-2-hydroxyethylcarbamonyl)-3-methyl-quinoxaline-*N*1,*N*4-dioxide) (Fig. 15) are the most known members of quinoxaline-1,4-dioxides, a group of synthetic antibacterial drugs. The proposed method constituted the first application of the MSPD technique in combination with hydrophilic interaction UHPLC to the determination of carbadox and olaquindox in animal feed. When compared to conventional reversed-phase HPLC separation, HILIC-UHPLC yielded a 5–10-times faster separation with an alternate selectivity. The developed MSPD method allowed extraction and clean-up to be carried out into a single step, without additional purification of the extracts.

In addition, the extraction procedure suggested was very simple, rapid and required only small sample and solvent amounts.

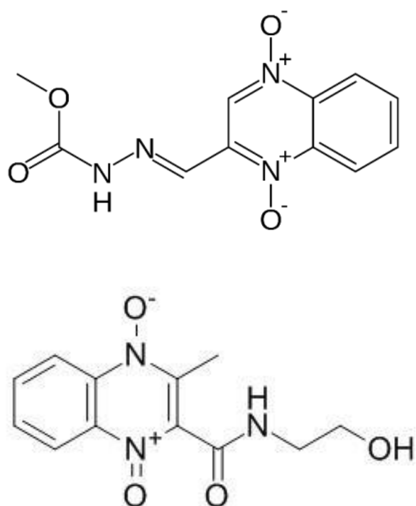


Figure 15. Carbadox (top) and olaquindox (bottom).

Recently, a convenient method was developed for simultaneous determination of 11 preservatives in cosmetics and pharmaceuticals. Matrix solid-phase dispersion had been optimized as the sample pretreatment technology. Experimental results showed that 11 preservatives were baseline separated within 22 min [94]. A simple, efficient, and sensitive strategy by coupling matrix solid-phase dispersion with ultra high performance liquid chromatography quadrupole time-of-flight mass spectrometry was proposed to extract and determine three types of components (including seven analytes) in Chinese patent medicines Chenxiangqu [95]. It was demonstrated that matrix solid-phase dispersion micro-extraction method could be enhanced efficiently using an ultrasound. Such method was established for the extraction and determination of multiple ingredients (psoralenoside, isopsoralenoside, psoralen, isopsoralen, bavachin, psoralidin, isobavachalcone and bakuchiol) from Fructus Psoraleae applying ultra-high-performance liquid chromatography [96]. The experimental factors affecting the extraction efficiency, including extraction solvent concentration, extraction solvent volume and ultrasound time, were optimized by the design of experiments methodology.

In general, a HPLC method based on matrix solid-phase dispersion (MSPD) was developed for simultaneous determination of numerous analytes in thousands of specimens.

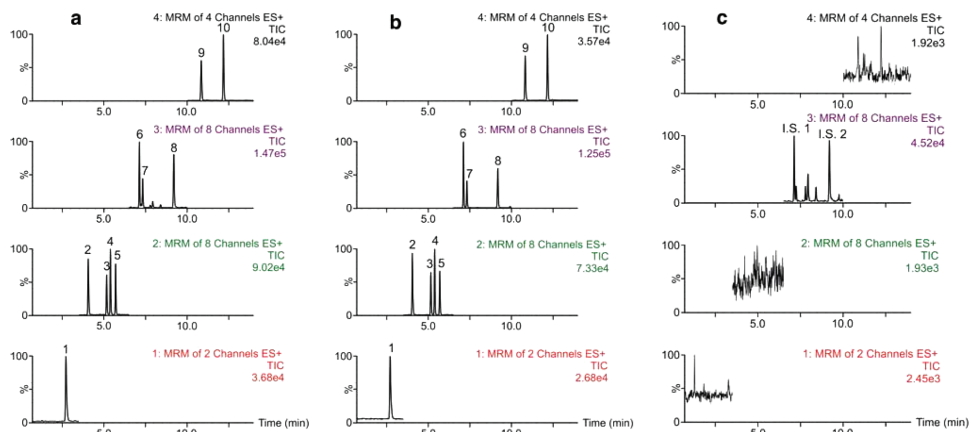


Figure 16. MRM UPLC–MS–MS chromatograms for a egg and b honey samples fortified with 5 μg kg^{-1} of each ten sulfonamides and c blank honey sample plus the two internal standards (5 μg kg^{-1} each). 1 = sulfacetamide; 2 = sulfadiazine; 3 = sulfathiazole; 4 = sulfapyridine; 5 = sulfamerazine; 6 = sulfamethazine; 7 = sulfamethizole; 8 = sulfamethoxazole; 9 = sulfabenzamide; 10 = sulfadimethoxine; I.S.1 = sulfamethazine-6C13; I.S.2 = sulfamethoxazole-6C13 [97].

Determination of ten sulfonamides (SAs) in egg and honey has been compared using column liquid chromatography (LC) and ultra-performance liquid chromatography (UPLC) coupled to tandem mass spectrometry (MS–MS) [97]. Fig. 16 shows the MRM UPLC–MS–MS chromatograms for egg and honey samples fortified with ten SAs.

In conclusion, on the basis of the obtained results it can be concluded that both UPLC and LC methods provide acceptable analytical performance but UPLC displayed shorter run-time, higher efficiency with better resolution and better precision, making this technique more suitable for confirmatory assay of SA residues.

Ultra-performance liquid chromatography-tandem mass spectrometry method still is using for the effective separation and determination of sulfonamides in milk [98], in water from tap, lake and river [99], in fruit juices [100] and other [101].

High-performance liquid chromatography on a glycopeptide antibiotic teicoplanin-based chiral stationary phase coupled with tandem mass spectrometry was developed for fast and reliable enantioseparation and determination of protein amino acids in hydrolyzed fertilizer samples [102].

Macrocyclic antibiotic based chiral stationary phases are compatible with both organic and aqueous mobile phases. Therefore, it was announced that Chirobiotic T2 could be the ideal stationary phase for resolving polar compounds, such as amino acids. Fig. 17 shows the plots of the retention times for three representative enantiomer pairs as a function of the MeOH concentration in the mobile phase. As seen, the retention times are substantially unaffected by changes in mobile-phase composition for MeOH concentrations lower than 30% (v/v). Obtained results suggested that, for very hydrophilic compounds, such as amino acids, hydrophobic interactions are not the driven retention forces even at water rich mobile phases. The method was validated in terms of linearity, limits of detection, limits of quantitation, precision, and accuracy. Linear responses were obtained with determination coefficients higher than 0.998 for all analytes, and limits of detection were from 0.04 to 0.24 µg/mL.

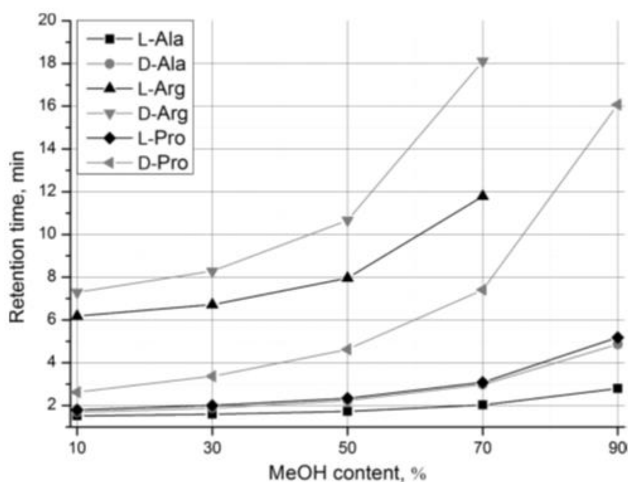


Figure 17. Effect of the methanol content in the mobile phase on the retention times of D-/L-Ala, D-/L-Arg, and D-/L-Pro enantiomers (n = 2) [102].

The developed method was successfully applied for determination of the free amino acid enantiomers in five commercially available hydrolyzed protein fertilizer samples. Later a reliable, sensitive and rapid method for the determination of nicotine and three minor alkaloids (cotinine, anabasine and nornicotine) in tobacco by ultra-high performance liquid chromatography in hydrophilic interaction chromatography mode coupled with tandem mass spectrometry (HILIC-MS/MS) has been established by the same authors [103].

Several review articles which cover the principles of the HILIC or HILIC-MS/MS methods, types of stationary and mobile phases, mechanisms of retention of highly polar compounds under HILIC conditions, implementation of 2D chromatography, and also fields of application of the methods have been published [104-109].

2.3. Types of columns used in gas chromatography

As it was mentioned earlier, in very beginning of gas chromatography columns were self made glass or metal tube filled with one or another substance particles. With evolution of gas chromatography the columns and its sorbents have been widely investigated and improved. The gas chromatography could be listed to two main parts according to the type of the column used: packed column and open tubular column. Packed columns represent part of columns used in gas chromatography where the whole column volume is filled with stationary phase. Open tubular columns represents part of columns used in gas chromatography where stationary phase are coated on the wall or on additional support structure and free passage are left for mobile phase in the middle of the column diameter. Comparison of some packed and open tubular columns are given in Table 2 [110].

Table 2. Comparison of characteristics of packed and open tubular columns

	Packed column	Open tubular
Outside diameter	3 – 6 mm	0.3 – 0.8 mm
Inside diameter	2 – 4 mm	0.25 – 0.53 mm
d_f	5 µm	0.1 – 1 µm
β	15 – 30	250 – 1000
Column length	1-2 m	15 – 60 m
Mobile phase flow	20-60 ml/min	1 ml/min
N	6000	180000
N_{eff}	2000/m	3000/m
H_{min}	0.5 mm	0.3 mm
Advantages	Lower cost Easier to make Easier to use Large sample loads Better for fixed gases	Higher efficiency Faster More inert More different applications could be applied Better for complex mixtures

2.3.1. Packed columns

Packed columns are typically 3 - 6 mm diameter and length of 0.6 - 2 m. Most commonly used material for column is stainless steel or glass. Packing material is a solid particles in case of gas - solid interaction type or solid particles coated with liquid stationary phase layer in gas – liquid interaction type. Particle size could vary from 60 to 150 µm. Over time, packed columns are being displaced by capillary columns from an increasing number of applications. However, there are areas of application where these columns still are used. This is due to several reasons. First of them is stability of liquid stationary phase. There are only few types of liquids what could be coated on the walls of capillaries and stay stable, while almost every low viscosity liquid could be coated on particles and used in packed columns. When the specific selectivity is necessary the packed columns with dedicated liquid stationary phase usually are used. Another reason of usage packed columns is quite high capacity of stationary phase could be accommodate in. It allows using these columns as preparative for collection of small amount of single components from initial mix. Third reason to use packed columns is adsorbents used in gas-solid interaction. Solid adsorbents cannot be coated and surface and it limits their usage in open tubular columns. Despite these limitations there is some modification of open tubular columns where some layer of porous material is coated on the column wall [111].

Stationary phase or packing material for classical packed columns in gas-liquid chromatography is prepared by coating solid particles with desirable liquid. Solid particles used as support should be inert to eliminate sample interaction with particle, to have large surface area, regular shape be mechanically stable and have to be a good conductor to heat. Most commonly used material for solid particles are diatomaceous earth. It is natural mineral widely spreaded in earth [112]. The typical chemical composition of diatomaceous earth is 80–90% silica, with 2–4% alumina (attributed mostly to clay minerals) and 0.5–2% iron oxide [113]. Liquid which will be used as a stationary phase is dissolved in an appropriate solvent, which should completely dissolve liquid stationary phase and must wet solid support. Then support particles are put into prepared liquid and mixed for a while until solid particles are saturated with the mobile phase solution. Then particles are dried by filtering and finally by evaporating solvent residues [114]. Dried particles are filled to empty column usually using vacuum. Most commonly used liquids for stationary phase could be listed as types of materials: hydrocarbons and perfluorocarbons; poly(siloxanes); ethers and poly(esters); ionic liquids; liquid crystals and chiral stationary phases.

Packing material for gas-solid chromatography is usually requires less preparation. Some uniform fractions of particles are prepared by filtering them through equivalent filter sets if necessary there is could be applied some deactivation procedures. Most commonly used sorbents are inorganic oxides, carbon adsorbents and molecular sieves. Most commonly used inorganic oxides are activated alumina and silica gel. These materials have quite high surface area and have relatively small specific activity toward organic samples. These salts have been used for general purpose analysis, also these materials could be modified with other inorganic salt to get some specific properties [115].

Carbon adsorbents represent wide class of materials where carbon is in various degree of graphite, crystalline carbon, carbon black, activated carbons and carbon molecular sieves. Carbon molecular sieves are used to separate light gases such as nitrogen oxides, carbon oxides, light hydrocarbons (C_1-C_3) [116].

Molecular sieves are class of aliuminosilicate materials also known as zeolites. These aliuminosilicates form crystaline structures with certain pore size. Most commonly used zeolites are Type 5A with pore size of 5 Å and 13X with pore size of 10 Å. The separation process has dual approach. Primary separation are controlled by molecular size. Smaller molecules can pass in pores of zeolite and their secondary process takes place which based on sorption energy of molecule to the surface. More polar molecules like water and carbon dioxide molecules are retained longer. Zeolites are used for separation permanent gases and light hydrocarbons [117;118].

2.3.2. Capillary (open tubular) columns

As mentioned earlier capillary columns are long and narrow tubes with stationary phase coated on the capillary wall or some support. Capillary columns could be divided to three main groups according to the nature of the coating material [119]:

1. Wall coated open tubular columns (WCOT). Stationary phase in these columns is a thin film of viscous liquid coated on the inner wall of the column. This type of gas chromatography columns are most common and conclude up to 80 % of all columns currently in use. These columns are with diameter from 100 up to 530 μm and from 15 up to 100 m length. Stationary phase film thickness in these columns vary from 0,1μm up to 5 μm.
2. Porous layer open tubular columns (PLOT). Stationary phase in these columns are fine solid particles of adsorbent. These particles are deposited

on the walls of the column with thickness from 5 up to 50 μm. These particles could be inorganic salts or organic polymeric materials. Column diameters vary from 0.2 to 0.6 mm and length from 2 m up to 30 m.

3. Support coated open tubular columns (SCOT). These columns contain a layer of fine support particles on the inner wall of the column coated with liquid stationary phase.

A fused silica is most commonly used as a frame for open tubular columns. This material was commonly appreciated from its introduction [120] because it's inertness toward solutes from nonpolar up to very strong polarity [121-123]. Before fused silica era there were used various types of materials for column frame along with various surface preparation techniques for deactivation of the surface of the column frame [124-128]. Most commonly glass and stainless steel were used. There were continuous activities to improve inertness of stainless steel [129]. Stainless steel annealed in a monosilane atmosphere is covered with a layer of silicon hydride. Inertness of the stainless steel capillary treated in such a way is very similar to the fused silica. Fused silica columns are drawn by specially designed machines at high temperature up to 2000 °C from prepared fused silica performs [130]. The preparation of stainless steel capillaries is much easier. It could be done by cold or hot rolling [130; 131].

Beside other qualities the stainless steel is conductive to electricity. This property is so far very little exploited in gas chromatography. Having in mind that the chromatographic process is held inside capillary and equation power in gas chromatography is increased by external heater. In nowadays gas chromatographs column are accommodated in an oven which is heated by some heaters. The construction has some disadvantages:

1. Column are heated through some additional media – air, which is not good heat conductor.
2. In case to rise up column temperature all surroundings should be heated up too, it requires additional heat consumptions and due to this increase inertness of system.

Using column made from stainless steel allows avoiding these disadvantages. Stainless steel capillary could serve not only as a frame for chromatography process but as well as heater too.

Most commonly used stationary phases in WCOT columns could be classified to four groups:

1. Nonpolar polysiloxanes. These polysiloxanes are most widely used in gas chromatography columns. Main of them are polydimethylsiloxane and polymethylphenylsiloxane. Varying the ratio of these polymers in the stationary phase could be received different mild-polarity stationary

phases. Most commonly used combination is 100 % polydimetylsiloxane known as -1 stationary phase and 5 % polymethylphenylsiloxane with 95 % polydimetylsiloxane known as -5 stationary phase [132]. Beside these two polymers also high interest is in polydimetylsiloxane modified by arylene in polymer backbone such modification gives lower stationary phase bleeding and preferred to use in application with mass spectrometry [133].

2. Functionalized polysiloxanes. Methyl groups in polydimetylsiloxane could be changed trifluoropropylmethyl, cyanopropylphenyl, bis-cyanopropyl, and some other functional groups to give a large range of possibilities to choose from.
3. Polar nonsiloxanes. These polymers are mainly based on polyethylene glycol (PEG) and similar materials called waxes. These polymers are very polars and usally used to separate very polar compounds like alcohols and acids. The lack of these materials is their relatively low thermal stability, 250 °C is upper limit for most of them.
4. Other hybrid materials. There are other polymeric materials whose do not fit to any above category. These materials like ionic liquids, liquid crystals or polycarboranes [134;135] have quite a different solubility properties neither polysiloxanes, but their efficiency or thermal stability properties require some special treatment.

2.4. Dyes and markers for use in fuel systems

According to the approval of the description of the order of the Ministry of Energy of the Republic of Lithuania regarding the marking of fuel subject to excise duty relief. Two types of dyes and one type of marker are used. Dyes are added to the fuel for house heating and (or) fuel for vehicles according to the intended use. If fuel is used for heating, then a red dye (Solvent Red - 19) and a yellow marker (Solvent Yellow - 124) are used. The coloring agent and marker used for heating fuels and their concentrations are described and indicated in Table 3.

Excise duty also does not apply to fuels containing blue solvent (Solvent Blue - 35) and yellow marker (Solvent Yellow - 124). The fuel for heating or fuel for vecihles so marked shall be used for agricultural operators, the production of agricultural products, aquaculture and commercial inland fishing enterprises and the marking of marine fuels. The dye and marker used and their concentrations are given in Table 4.

Table 3. Labeling of the fuel for heating [136]

No.	Marking materials			
	Name	Purpose	Colour	Min dosing c (mg/kg),
3.1	„Solvent Red - 19“ N-Ethyl-1-((4-phenyldiazenyl)phenyl)diazenyl)naphthalen-2-amine	Dye (one of the selected dyes is used for dyeing)	Red	5.0
3.2	N-(2-ethylhexyl)-1-[[2-methyl-4-[(2-methylphenyl)azo]phenyl]azo]naphthalen-1-amine	Dye	Red	6.5
3.3	1-[[2-methyl-4-[(2-methylphenyl)azo]phenyl]azo]-N-tridecylnaphthalen-2-amine	Dye	Red	7.4
3.4	„Solvent Yellow - 124“ N-Ethyl-N-[2-[1-(2-methylpropoxy)ethoxy]ethyl]-4-phenyldiazenylaniline	Marker	Yellow	7.11–10.66

Table 4. Labeling of fuel for vehicles intended for use by agricultural operators for the production of agricultural products, aquaculture and commercial fishing in inland waters and marine fuels [136].

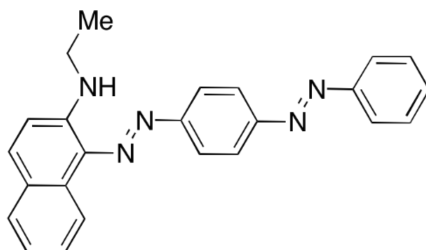
No.	Marking materials			
	Name	Purpose	Colour	Min dosing c (mg/kg)
4.1	„Solvent Blue - 35“ 1,4-bis(butylamino)anthraquinone	Dye (other blue 1,4-bis (alkylamino) - 9,10 anthraquinone dyes may be used in a concentration such that the color intensity of the dyed fuel corresponds to that given by the standard dye)	Blue	4.2
4.2	„Solvent Yellow - 124“ N-Ethyl-N-[2-[1-(2-methylpropoxy)ethoxy]ethyl]-4-phenyldiazenylaniline“	Marker	Yellow	7.11–10.66

According to this order, the dyes and markers must have the following properties:

1. Fuels shall be labeled with a dye and a tracer or a mixture thereof that is soluble in the fuel and does not react with it.
2. Dyes from different manufacturers with the same chemical formula may have different brand names.
3. Dissolved tracers must be visible and/or determined by chemical tests.

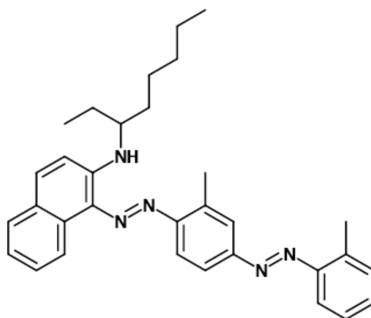
2.4.1. Structure and properties of dyes and markers:

1. Solvent Red – 19
N-Ethyl-1-((4-phenyldiazenyl)phenyl)diazenyl)naphthalen-2-amine

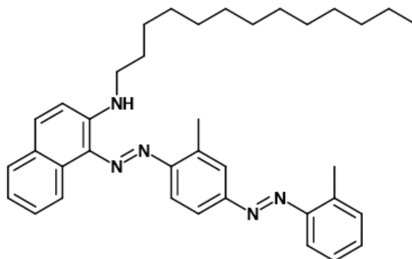


The basic structure of the red dye consists of aminonaphthalene with an ethyl group attached to the amine in the first position and a phenylazophenylase group in the second position. It is also possible to have two substituents when an ethylhexyl or tridecyl group is attached to the amino group in the first position and tolylazotolylase group is attached to the amino group in the first position instead of phenylazophenylase.

2.N-(2-ethylhexyl)-1-[[2-methyl-4-[(2-methylphenyl)azo]phenyl]azo] naphthalen-1-amine

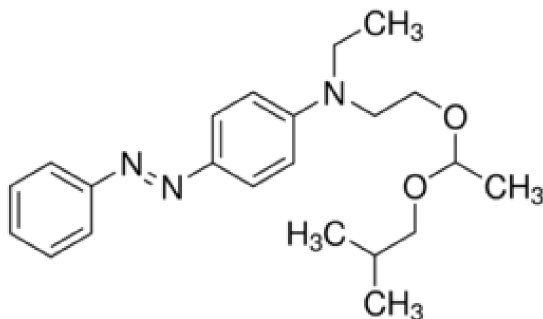


3.1-[[2-methyl-4-[(2-methylphenyl)azo]phenyl]azo]-N-tridecylnaphthalen-2-amine



Dyes 1.2 and 1.3 are also red and can be used for marking fuel for vehicles and (or) fuel for heating according to the order of the Ministry of Energy of the Republic of Lithuania. They are interchangeable (Solvent Red - 19), but must be used in certain concentrations as shown in Table 4.

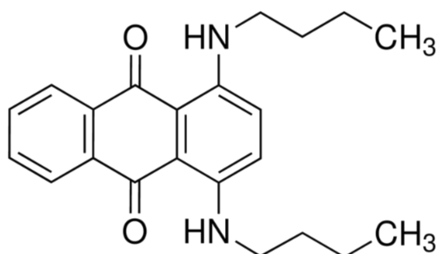
4.N-Ethyl-N-[2-[1-(2-methylpropoxy)ethoxy]ethyl]-4-phenyldiazenylaniline



The basic structure of the yellow marker consists of aniline, in which one hydrogen in the amino group is replaced by an ethyl group, the other hydrogen is replaced by an isobutyloxyethyloxyethyl group and the phenylazo group is attached in the 4 - position of the aniline.

5. Solvent Blue – 35

1,4-bis(butylamino)anthraquinone



The base of the blue dye is an anthraquinone to which a butylamine group is attached on one side at positions 1 and 4.

2.4.2. Overview of detection methods

Many methods for determining dyes and markers for heating fuels and automotive fuels can be found in the literature. There are many dyes and markers for fuels in the world, and almost every country that produces fuels for domestic use and trades in other countries has certain dyes and markers to distinguish between the uses for which fuels have been produced. Due to the abundance of different dyes and markers, there are various methods of qualitative and quantitative analysis. Huang and coworkers [137] described an electrochemical method for determining the orange colour (Solvent Orange - 7) in a fuel. The orange dye is oxidized with Britton-Robinson buffer (0.04 M H_3BO_3; a mixture of 0.04 M H_3PO_4 and 0.04 M CH_3COOH is titrated to the required pH with 0.2 M NaOH) and N,N-dimethylformamide in a 1: 1 mixture (v/v). integral). Carbon and silver chloride electrodes are immersed in the resulting mixture. An excellent analytical signal is obtained at a voltage of +0.70 V. This signal can be monitored by linear-scan voltammetry (LSV) or square-wave voltammetry (SWV). Using the square wave voltammetric method, the orange dye can be detected at a concentration of $4.0 \cdot 10^{-6}$ to $18.0 \cdot 10^{-6}$ mol/L using a calibration curve with a linearity of 0.995. Brzozka [138] has described a voltammetric method that detects a blue dye (Solvent Blue - 14) in a fuel. These analyzes are based on the use of a flat printed carbon electrode (SPCE). The test solution is prepared by mixing a sample of the fuel to be analyzed with a pre-prepared 1: 1 mixture of Brittom-Robinson buffer with N, N-dimethylformamide (one volume by volume) to which is added $5.50 \cdot 10^{-4}$ mol/L bis (2-ethylhexyl) sodium sulphoxide salts (DSS - dioctyl sulfosuccinate sodium). Using this method, the analytical signal is obtained at a voltage of -0.40 V. Under the best conditions, a calibration curve can be established showing the concentration of blue dye from $2.00 \cdot 10^{-7}$ mol/L to $2.00 \cdot 10^{-6}$ mol/L, the linearity of the calibration curve being 0.9986 and the detection limits of the method being $9.30 \cdot 10^{-8}$ mol/L. Goldman [139] describes the spectrophotometric analysis of red dyes in fuel. Spectral data are recorded at 535 nm, at the maximum absorption of the red dye. These analyzes are performed on standard red dye solutions of known concentrations. Initially, standard red dye solutions of different concentrations are prepared to measure their absorbances at 535 nm and to form a calibration curve. Then, after the addition of the appropriate red dye from which the calibration curve

has been drawn, the samples are analyzed and the results obtained compared with the calibration curve. This calculates the concentration of a particular red dye in the fuel used. Timkovich [140] described how a radon dye (Solvent Red - 26) can be determined using a gas chromatograph with a mass spectrometer. A gas chromatograph with a model of HP5890GC was used in the study. Helium gas is used as the mobile phase, the flow rate of the mobile phase is 3.7 ml/min. A short 3 m capillary column with an internal diameter of 0.25 mm is used. The stationary phase of dimethylpolysiloxane is used in the column. Before testing the fuel sample with the red dye, it is diluted with acetone to a concentration of 0.5 ng/μL to 1.5 ng/μL required for injection. 1 μL of test sample is injected into the system. Initially, the temperature is maintained at 160 ° C for 0.5 minutes, then raised to 300 ° C using a step of 45 ° C/min, when the required temperature is reached, it is maintained until the end of the analysis. Analysis under these conditions gives a peak in the chromatogram at 4.20 minutes. After estimating the sample dilution, calculate the concentration of red dye in the test sample.

Agilent technologies described [141] how to determine new fuel markers using a 2-dimensional gas chromatograph with a mass spectrometer (2-Dimensional GCMS). The essence of these analyzes is to detect as little new tracers as possible in the fuel, without using any sample preparation techniques, to inject the test sample directly into the analytical system. An 7693A auto-liquid sampler, a 7890B gass chromotograph, a flame ionization detector (FID) and a 5977A mass spectrometric detector (GC) are used for this analysis. MSD - mass spectrometer detector). The fuel marker used is detected by electron impact ionization and selected ion monitoring (SIM). Two analyzes are performed, one of which is an unlabeled fuel and the other the same fuel with some marker, they are compared with each other. The detection limit of this system is from 25 ppb to 2.5 ppm (1 ppm = 10^{-6}; 1 ppb = 10^{-9}) of the concentration of dye used in the fuel. Tomič and Nasipak [142] proposed a methodology for the detection of blue dye (Solvent Blue - 35) and yellow marker (Solvent Yellow - 124) using normal phase liquid chromatography with UV / DAD detector. This procedure was performed with an Agilent 1100 liquid chromatography system. The module of this system consists of a high-pressure pump, an automatic sample injection system, a separating column packed with silica gel (Lichrosorb SI 60, 5 mm, 25x0.4 cm), a UV / DAD detector and a computer with control software that collects the received data. The mixture used in the mobile phase consists of: toluene and ethyl acetate in a ratio of 98:2 (%, v/v). Wavelengths of 650 and 450 nm are used for analyte detection. These wavelengths are selected based on the maximum absorption of the analytes. The maximum absorbance of the blue dye (Solvet Blue - 35)

is at 650 nm and the maximum absorbance of the yellow marker (Solvent Yellow - 124) is obtained at 450 nm. The chromatograms show the retention times of the substances sought: 8.3 minutes for the blue dye and 10.7 minutes for the yellow marker. In order to determine the concentrations in the real samples, calibration curves are first drawn up, after which the real samples are allowed. Samples must be passed through a 0.45 micron filter to remove all possible impurities before samples are introduced for analysis. After filtration, the sample can be applied to the column and analyzed. The results of the real samples obtained are compared with the calibration curves and the exact concentrations of the dye and tracer used are calculated.

DIN 51430 [142] - (German Deutsches Institutut fur Normung) German Institute for Standardization standard no.: 51430. It describes a method for normal phase liquid chromatography that allows the detection of a wide variety of fuel for heating and fuel for vehicles dyes, including the red dye (Solvent Red - 19) and the yellow dye (Solvent Yellow - 124). This standard specifies method for the quantitative determination of dyes and markers in the range up to 10 mg/ml.

3. EXPERIMENTAL PART

3.1. Reagents and solutions

Pentane (99%), hexane (99%), isopropyl alcohol (99%), nitric acid (70%) dichloromethane (99.8%), n-octane (99%), n-nonane (99%), n-decane (99%), n-dodecane (99%), n-tridecane (99%), n-tetradecane (99%), benzene (99%), chlorobenzene (99.9%), pentyl acetate (99%), cumene (isopropylbenzene) (98%), aniline (benzenamine) (99.5%), 1-hexanol (98%), 2-butanone (99%), propionic acid (99%), dimethylpolysiloxane (PDMS) (99%), toluene (HPLC grade), hexane (HPLC grade), MTBE (methyl tret-buthyl ether) (99.8%), Solvent Red 19 (N-ethyl- 1-(phenyldiazophenylazo)-2-aminonaphtalen) (96%), Solvent Blue 35 (1,4-bis(butylamino)-9,10-anthraquinone) (98%), Solvent Yellow 124 (N-ethyl-N-[2-(1-izobutoxyethoxy) ethyl]-4-phenildiazenyl) aniline) (98%) were purchased from Sigma-Aldrich (Germany). Ethyl acetate (HPLC grade) was purchased from Merck (Germany).

Stock solutions of individual compounds (n-octane, n-nonane, n-decane, n-dodecane, n-tridecane, n-tetradecane, benzene, chlorobenzene, pentyl acetate, cumene, aniline, 1-hexanol, 2-butanone and propionic acid (10 mg ml^{-1} each)) were prepared in dichloromethane. Six working solutions were prepared in dichloromethane:

1. Benzene, n-octane, chlorobenzene, pentyl acetate, cumene and aniline (1 mg ml^{-1} each).
2. 2-Butanone and n-octane (1 mg ml^{-1} each).
3. 1-Hexanol and n-octane (1 mg ml^{-1} each).
4. Propionic acid and n-octane (1 mg ml^{-1} each).
5. n-Octane, n-nonane, n-decane, n-dodecane, n-tridecane and n-tetradecane (1 mg ml^{-1} each).
6. n-Decane (1 mg ml^{-1}).

Stock solutions of the fuel dyes and the marker (SR 19, SB 35 and SY 124) (10 mg/L each) were prepared in toluene. Working solutions of the fuel dyes and the marker were prepared by dilution of the stock solution with toluene. All solutions were stored in the dark at 4°C. Dyed fuel samples were purchased from the local petrol stations.

3.2. Fabrication methods

3.2.1.Stainless steel capillary column preparation

Capillary column frame preparation

The 304 grade stainless steel capillary of 0.5 mm OD and 0.32 mm ID has been acquired from local suppliers. 30 m of capillary was cut from bulk coil and winded on 17 cm radius frame.

Capillary column preparation before coating

Freshly prepared capillary was connected to Elmer S200 liquid chromatography pump and washed 30 min with 0.5 ml/min hexane flow rate to remove residual oils from capillary manufacturing.

After washing with hexane, capillary have been washed in same way with acetone and isopropyl alcohol.

Finally capillary have been washed with deionizer water.

Stainless steel passivation

To mask active ferrous atoms in stainless steel surface, capillary have been washed with nitric acid 20 % HNO_3 solution in water. During passivation procedure capillary column have been accommodated in 60°C thermostat and nitric acid solution pumped through the column at 0.1 ml/min flow overnight (at least 12 h). After passivation column have been washed with deionizer water until pH value become 7.

Stainless steel capillary coating techniques

There two coating techniques have been used for preparation of column: dynamic and static.

During dynamic coating procedure freshly prepared coating solution was filled to the syringe and syringe fixed in the Hamilton syringe pump. Capillary have been connected to the syringe needle through dedicated adapter and solution has been pumped through the capillary with 0.1 ml/min flow. After capillary have been filled with the coating solution syringe have been filled with air and connected back to the capillary and same 0.1 ml/min flow rate have been used to push out the excess of the coating solution. Only small piece of the coating solution left on the walls on the column. Thickness of the coating was proportional to the amount of the PDMS in the coating solution.

After excess of the coating solution has been removed the prepared column connected to the injector port of a gas chromatograph. Carrier gas pressure has been set in the injector to 0.8 barg and carrier gas has been allowed to pass through the column overnight or at least 12 hours at ambient temperature to let dry out residues of solvent. After initial drying another end of column has been connected to FID detector. Temperature program of the oven of GC has been set to rise up temperature from 35°C to 330°C with 1°C/min temperature ramp. After oven temperature reach its final value column has been left in that temperature until the signal value written by the FID detector stabilized in flat plateau.

During static coating procedure capillary have been filled with coating solution in same way as in the dynamic coating. After capillary have been filled with coating solution, both ends of capillary have been connected to T connector, third end of the T connector through the same capillary have been connected to the vacuum pump. Vacuum pump has created 0,4 bar absolute pressure. Capillary with coating solution have been accommodated to the thermostat and the thermostat temperature have been set close to the boiling point of the solvent in which have been dissolved PDMS. Vacuuming of the capillary has been performed until all liquid phase evaporated. Typically process takes from 6 up to 12 hours. After evaporation of the solvent column has been connected to the GC injector port and the FID detector. Final drying and conditioning of the column has been performed in same way as in the dynamic coating case.

PDMS coating solution preparation

Coating solution for the dynamic coating has been performed by diluting PDMS oil with hexane with ratio from 1:4 up to 1:10. Diluted solution has been placed in ultrasonic bath for 10 min to reach homogenous mixture.
Coating solution for the static coating has been performed by adding accurately calculated mass of the PDMS oil to pentane. Necessary amount of PDMS oil has been calculation has been made having several assumptions:

a) Capillary inner surface has been treated as an ideal cylinder
b) PDMS oil has not evaporated and have not been drawn out from the column by vacuum together with solvent
c) PDMS oil covered the entire surface with uniform layer.

As a starting point has been selected 0.25 μm layer thickness.

The necessary amount of the PDMS oil was calculated by calculating the volume of the uncovered capillary and subtracting volume of the cylinder covered with 0.25 µm coating layer.

3.2.2. Standard solutions for analysis of fuel dyes and their preparation

Certain, weighted, amount of automotive fuel dyes („Solvent Blue – 35“ ir „Solvent Red – 19“) and marker („Solvent Yellow – 124“) has been diluted in toluene. Several dilution steps have been performed to achieve necessary concentration level. Dilution has been performed with HPLC grade toluene. Standard solution preparation data are listed in Table 5.

Table 5. Standard solutions and dilution levels

Solvent:	**Density (g/ml):**
Toluene	0,867

First dilution level:				
Dyes:	**Mass of container (g):**	**Mass of dye (g):**	**Toluene mass (g):**	**Volume of toluene (ml):**
„SR – 19“	14,57572	0,00136	8,70252	10,0375
„SY – 124“	14,76953	0,01163	8,65990	9,9884
„SB – 35“	14,38766	0,00265	8,66853	9,9983

„Standards v1“		
Dyes:	**Dye conc. (mg/L):**	**Dye conce. (mg/mL):**
„SR – 19“	135,0229	0,1350
„SY – 124“	1160,3275	1,1603
„SB – 35“	264,1278	0,2641

Solvents marked in Table Standards v1 have been used to prepare intermediate dyes („Solvent Blue – 35“ and „Solvent Red – 19“) and marker („Solvent Yellow – 124“) solutions with concentrations 20 mg/L of each dye. Final standard solutions for calibration have been prepared by diluting calculated amount of intermediate solution up to 2 ml with toluene. Calibration solutions for calibration curve data are listed in Table 6.

Table 6. Solvents for dyes and markers calibrations curves

Dye	Cal. lev.	Dye conc. (mg/L)	Amount of intermediate solution (L)	Mass of intermediate solution (g)	Toluene (g)
„SR – 19“	1	1	0,0001	0,0867	1,6473
„SB – 35“	1	1	0,0001	0,0867	1,6473
„SY – 124“	1	1	0,0001	0,0867	1,6473
„SR – 19“	2	3	0,0003	0,2601	1,4739
„SB – 35“	2	3	0,0003	0,2601	1,4739
„SY – 124“	2	3	0,0003	0,2601	1,4739
„SR – 19“	3	5	0,0005	0,4335	1,3005
„SB – 35“	3	5	0,0005	0,4335	1,3005
„SY – 124“	3	5	0,0005	0,4335	1,3005
„SR – 19“	4	7	0,0007	0,6069	1,1271
„SB – 35“	4	7	0,0007	0,6069	1,1271
„SY – 124“	4	7	0,0007	0,6069	1,1271
„SR – 19“	5	10	0,0010	0,8670	0,867
„SB – 35“	5	10	0,0010	0,8670	0,867
„SY – 124“	5	10	0,0010	0,8670	0,867

Solution for calibration curve concentration verification:					
Calibration level:	1	2	3	4	5
Conc. of standard solution (mg/L):	1	3	5	7	10
Container mass (g):	2,7799	2,7520	2,7448	2,7174	2,7499
Mass with standard (g):	2,8667	3,0201	3,1856	3,3164	3,6190
Mass with toluene (g):	4,5226	4,4841	4,4693	4,4428	4,4950
Standard mass (g):	0,0868	0,2681	0,4408	0,5990	0,8691
Toluene mass (g):	1,6559	1,4641	1,2837	1,1264	0,8760
Standard and solvent mass (g):	1,7427	1,7322	1,7245	1,7254	1,7451
Total volume (mL):	2,01	2,00	2,00	2,00	2,02
Standard solution conc. (mg/L):	1,00	3,10	5,11	6,94	9,96

For the normal phase chromatographic analysis as a solvent have been used toluene, hexane, ethylacetate, methyltretbutyleter.

3.2.3. Sample preparation for dyed fuel

The real samples of dyed fuel were filtered through 0.45 μm PTFE Chromafil Xtra PTFE-45/25 filters (Macherey-Negel, Germany) filters. After filtering, the samples were transferred into 2 mL chromatographic vials.

3.3. Techniques

3.3.1. Gas chromatographic conditions

The chromatographic analysis was performed on a Shimadzu GC-2010 Plus gas chromatograph equipped with an AOC-20i auto injector and a flame ionization detector. The following gas chromatographic columns were used: PerkinElmer fused silica Elite-1 (crossbond 100% dimethyl polysiloxane) capillary column (30 m × 0.32 mm ID, film thickness 1 μm) and stainless steel 100% dimethyl polysiloxane capillary column (30 m × 0.32 mm ID) prepared in our laboratory. Helium was employed as a carrier gas with a column flow rate of 1.2 ml min^{-1}. The injector temperature was held at 250 °C. Injection was performed in a split mode with a split ratio of 10:1. The flame ionization detector temperature was held at 250 °C. Helium gas was used as make up gas at 30 ml min^{-1} flow rate. The hydrogen flow rate was 40 ml min^{-1}, the air flow rate was 400 ml min^{-1}. The oven temperatures were as follows: 1) for working solutions 1, 2 and 6 the oven temperature was 100 °C; 2) for working solutions 3 and 4 the oven temperature was 60 °C, and 3) the temperature programmed mode was used for solution 5: 170 °C for 1 min, from 170 to 200 °C at 7 °C min^{-1} and held at 200 °C for 3 min.

3.3.2. Parameters of HPLC analysis

The chromatographic analysis was performed on a Liquid Chromatograph PerkinElmer Series 200 (PerkinElmer Instruments, USA) using UV/Vis spectrophotometric detection. A 200 μL volume injection loop was used for automated injection. The HPLC system was equipped with a Zorbax Rx-SIL column (250 mm × 4.60 mm ID, sorbent particle size 5 μm) (Agilent Technologies, USA). A 150 μL high pressure mixer (PerkinElmer Instruments, USA) was used. Real samples were filtered through a 0.45 μm

PTFE filter right into a 2 mL vial. The vials with the prepared samples were placed into an autosampler for injection. The best separation of analytes with the shortest retention times was achieved while using a mobile phase consisting of hexane, toluene and ethyl acetate. A constant mobile phase flow of 1 mL/min was set. A gradient composition of the mobile phase was used to get the best results: firstly, 7 min the column was conditioned with a hexane/toluene (30/70 vol.%) mixture. The first 2 minutes after the injection the mobile phase composition was the same as used for preconditioning; after 8 min the mobile phase composition changed to the toluene/ethyl acetate (93/7 vol.%) solution; in the last step the isocratic mobile phase composition was held for 5 min. The total time of the analysis of dyes and markers in fuel was 22 min. The HPLC column must be preconditioned for 7 min before every analysis to remove ethyl acetate from the column.

4. RESULTS AND DISCUSSIONS

4.1. Development and assessment of the in-house made stainless steel capillary GC column

4.1.1. Optimisation of mobile phase flow rate

Column efficiency depends on the mobile phase flow rate. At the optimal flow rate the theoretical plate height is smallest. Thus, first of all optimal mobile phase flow rate through a laboratory prepared column and commercial PerkinElmer *Elite-1* column was determined. The working standard solution of n-decane in dichloromethane was used. Helium flow rate varied from 0.5 to 7 ml min^{-1} and the theoretical plate height H was calculated employing equations [23] and [24]:

$$\mathrm{N} = \left(\frac{t_r}{\frac{w_h}{2.35}}\right)^2 = \left(\frac{2.35 t_r}{w_h}\right)^2 = 5.54\left(\frac{t_r}{w_h}\right)^2 \tag{23}$$

and

$$\mathrm{H} = \frac{L}{N} \tag{24}$$

where N is a number of theoretical plates, t_R is the retention time of n-decane, $w_{0.5}$ is the peak width (at 0.5 of height) of n-decane, L is the column length.

As can be seen from the results presented in Fig. 18, for the *in-house* developed stainless steel column as well as for *Elite-1* the optimum mobile phase flow rate is 1.2 ml min^{-1}.

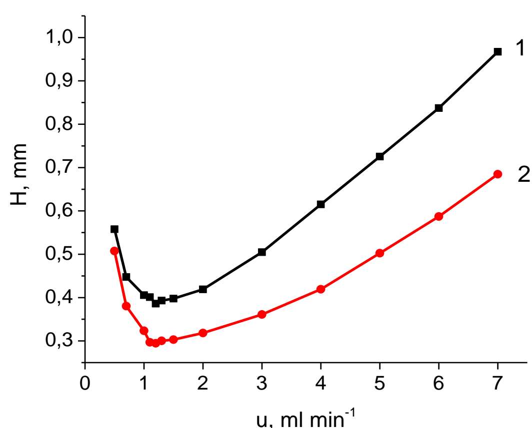


Figure 18. Effect of mobile phase flow rate on plate height for *in-house* made (1) and *Elite-1* (2) columns.

The results also demonstrate that commercial *Elite-1* is more efficient with the minimal plate height of 0.295 mm, meanwhile for our prepared column the minimal H is 0.386 mm. The both columns were coated with the same stationary phase PDMS and in the both cases the same carrier gas helium was used. Internal diameter of *Elite-1* is 0.25 mm however our column has 0.32 mm ID. This could be the reason of better efficiency of *Elite-1*. One more probable reason of the difference in the efficiencies of the columns is a less uniform stationary phase film on stainless steel column.

The computational investigations of the hydrodynamics of counter current flows in a structured packed column are multiscale probe [144]. As expected, the interfacial area initially increases with increased liquid load and then remains un-affected. The effect of the surface characteristics on the wetting and spreading is studied using the contact angle boundary condition at the corrugated sheets. Contact angle is one of the critical factors that dictates the wetting, and thereby the interfacial area.

4.1.2. Test probe selection

The activity of a column is determined by the measurement of any deleterious effects the column has towards challenging compounds. These interactions may be acidic, basic, or strongly hydrogen bonding. Poor behaviour is exhibited by tailing peaks or reduced peak response. Both of these behaviours lead to inaccurate calculation of the peak areas and consequently inaccurate quantification of the active compounds of interest [145].

Probes selected for column testing should demonstrate if the column is thermally stable, properly deactivated, if it contains the correct amount of a stationary phase.

As it was mentioned in the introduction section, the choice of the individual compounds in the test probe can either highlight or mask the deficiencies of a column with respect to activity. By selecting undemanding probes, column activity can go undetected. Ideally test compounds should be molecules with low molecular weights, low boiling points, and no steric shielding of the active groups. These characteristics allow the probative portion of the test molecule to penetrate and fully interact with the column's stationary phase and surface [145].

For GC column evaluation Grob test mixture is available commercially. However, Grob test mixture is a quite undemanding probe. For example, it contains 2,6-dimethylphenol as an organic acid to test column basicity and 2,6-dimethylaniline as a base to test column acidity. Those compounds are

weak probes as the active sites of the molecules are shielded by the two methyl groups on the phenyl ring.

In order to prepare a more demanding probe we examined more than 30 test probe candidates and selected nine compounds, namely benzene, n-octane, chlorobenzene, pentyl acetate, cumene, aniline, 2-butanone, 1-hexanol and propionic acid. In order to elute the compounds from the column in a reasonable time but, on the other hand, to use as low column temperature as possible and to avoid peak overlapping, four different mixtures containing those compounds were prepared (Table 7). 2,6-Dimethylaniline from the Grob test mixture was substituted by aniline and 2,6-dimethylphenol was substituted by propionic acid. The molecules of aniline and propionic acid have no steric shielding of the active groups thus can fully interact with the column‘s stationary phase and surface. Also, in order to make the test more demanding, we substituted 1-octanol by 1-hexanol. As the chain length in the alcohol decreases, the molecules become less hydrocarbon-like and thus more active. At elevated temperatures the interactive forces of the analytes are diminished, thus weakening their usefulness as test probes [145]. Taking this under consideration and seeking to prepare a demanding test, methyl esters of decanoic, undecanoic and dodecanoic acids from the Grob test mixture were substituted by pentyl acetate.

Table 7. Test mixtures in dichloromethane

Mixture	Compounds (10 mg ml^{-1} each)
1	benzene, n-octane, chlorobenzene, pentyl acetate, cumene, aniline
2	2-butanone, n-octane
3	1-hexanol, n-octane
4	propionic acid, n-octane

4.1.3. Column inertness evaluation

The inertness of the column was evaluated based on peak shapes of the test mixtures analytes. When the column was evaluated using the mixture 1, good peak shapes were observed only for nonpolar analytes (Fig. 19a). The peaks of benzene, n-octane and cumene were symmetric and comparable with those obtained on the commercial column *Elite-1* (see Table 8 and Fig. 19).

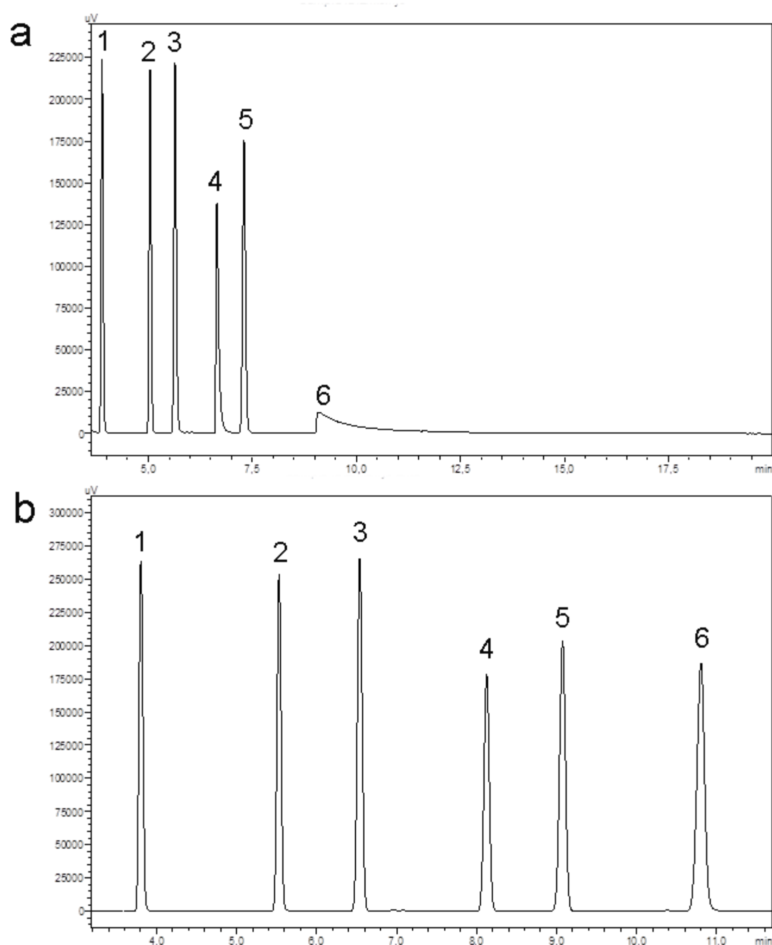


Figure 19. Chromatograms of the mixture 1obtained using *in-house* made stainless steel column (a) and *Elite-1* (b). 1 - benzene, 2 - n-octane, 3 - chlorobenzene, 4 - pentyl acetate, 5 - cumene, 6 –aniline. Chromatographic conditions are described in Experimental.

With the increase of compounds polarity, peak asymmetry increased to 1.17 for slightly polar chlorobenzene and to 2.6 for more polar pentyl acetate. Tailed peaks of polar molecules indicate a possible dipole-dipole interaction of the analytes with the column walls. Like esters, ketones are polar molecules and thus also interact with the column walls as it is evident from the peak of 2-butanone (Fig. 20a and Table 8). A very poor performance was observed for aniline. Aniline peak tailing at 10% height was 11. Aniline is a basic analyte thus it‘s tailing peak indicates the presence of acidic active sites in the column. Aniline is able to form hydrogen bonds, thus its deteriorated peak is an evidence of a probable presence of hydrogen-bonding sites in the column.

Table 8. Peak tailing measured at 10% of peak height

Compound	*In-house* column	*Elite-1*
Benzene	1.10	1.05
n-Octane	1.06	1.02
Chlorobenzene	1.17	1.01
Pentyl acetate	2.60	0.99
Cumene	1.08	0.97
Aniline	11.0	0.94
2-Butanone	3.01	1.11
1-Hexanol	11.1	0.99

Very asymmetric peak of 1-hexanol also could be explained by hydrogen bond formation with the active sites in the column (Fig. 21a and Table 8). Propionic acid was particularly strongly retained in the column and its peak was not observed in the chromatogram. In comparison, the peaks of the analytes obtained on the commercial column *Elite-1* at the same chromatographic conditions were symmetric (see Fig 19b, Fig. 20b, Fig. 21b and Table 9).

Recently published work [146] presents a novel column fabrication approach for capillary gas chromatography (CGC) through the condensation reaction of a hydroxyl-terminated polymer with a cross-linker inside a capillary column. In this work, polypropylene glycol (PPG 4000) and tris[3-(trimethoxysilyl)propyl]isocyanurate (ICS) were employed to fabricate the PPG-gel capillary column for GC analyses. Inside the column, a 3D cross-linked organogel network forms on the capillary inner surface by condensation of PPG 4000 with ICS under the given mild conditions. As a result, the PPG-gel column exhibited moderate polarity and showed distinctly advantageous resolving performance and inertness for carboxylic acids over the PPG column by the conventional static coating method. Also, it achieved high-resolution separations of the isomer mixtures of alkanes, alkylbenzenes and halobenzenes. Moreover, the PPG-gel column exhibited dramatically improved solvent stability because of its cross-linked organogel network. The organogel column displayed excellent column repeatability and reproducibility with the RSD% values in the range of 0.01-0.04% for run-to-run, 0.71-0.84% for day-to-day and 2.4-3.1% for column-to-column.

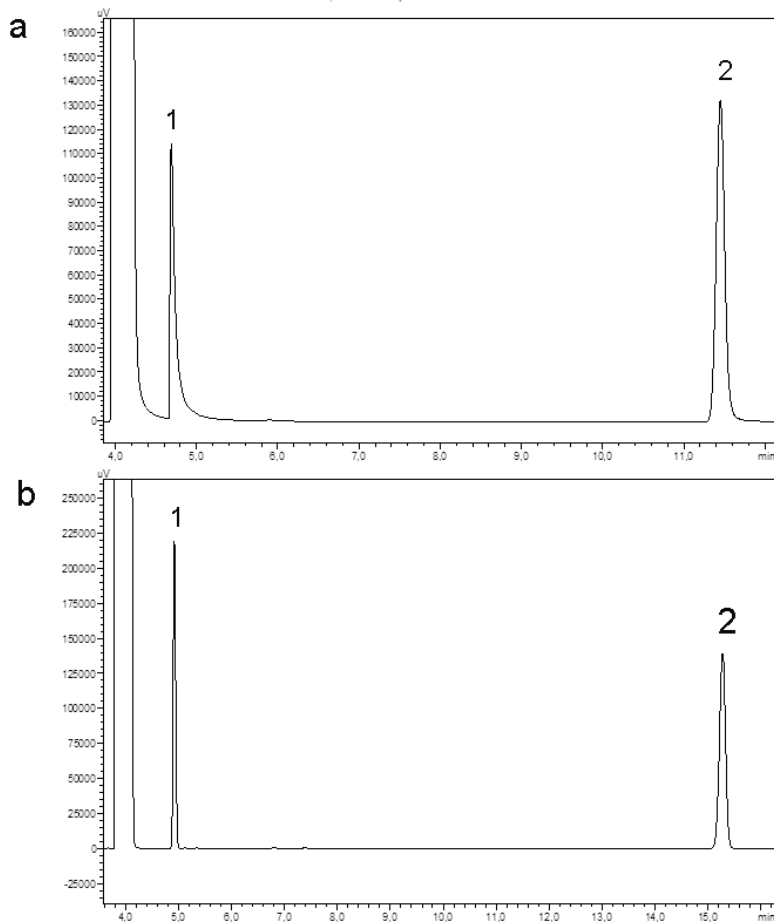


Figure 20. Chromatograms of the mixture 2 obtained using *in-house* made stainless steel (a) and *Elite-1* (b) columns. 1 - 2-butanone, 2 - n-octane. Chromatographic conditions are described in Experimental.

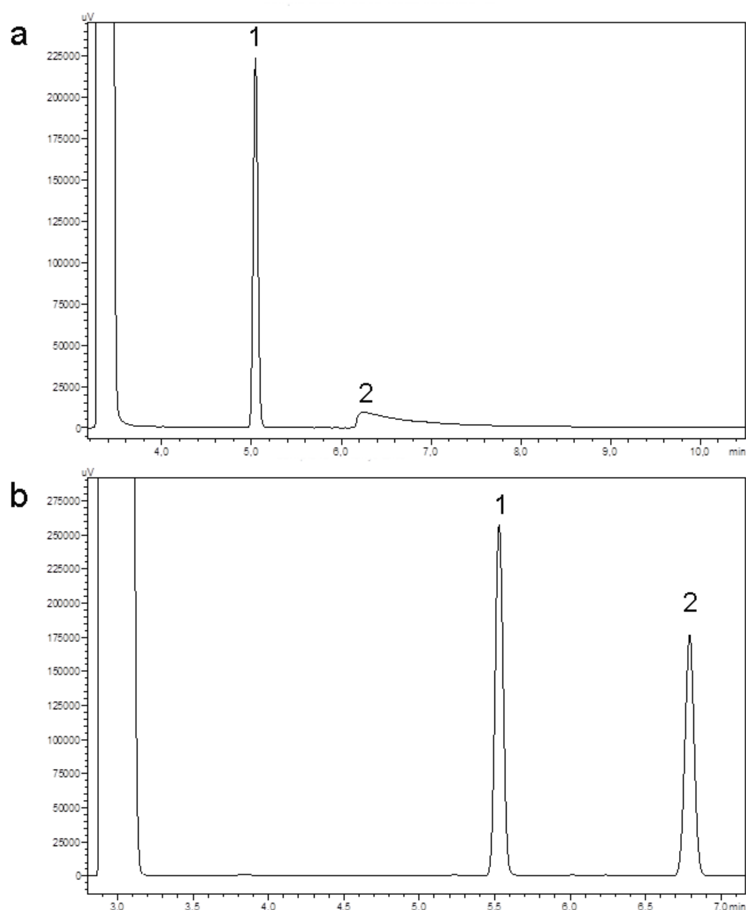


Figure 21. Chromatograms of the mixture 3 obtained using *in-house* made stainless steel (a) and *Elite-1* (b) columns. 1 – n-octane, 2 – 1-hexanol. Chromatographic conditions are described in Experimental.

The proposed our approach is facile and applicable to other analogous materials and has a promising future for wide applications.

4.1.4. Column longevity evaluation

The column‘s thermal longevity test was accomplished after conditioning the column from 1 to 40 hours at 200°C. The column longevity was characterised by the loss of responses of the active analytes and the shift of retention times [147].

Re-testing of the column after conditioning revealed that the retention times shortened. It is particularly evident for later eluting peaks, e.g. for n-octane in the chromatogram of the test mixture 3 (Fig. 22). n-Octane retention

time decreased from 11.45 min (1 hour conditioning) to 11.38 min (40 hours conditioning) indicating a significant stationary phase loss.

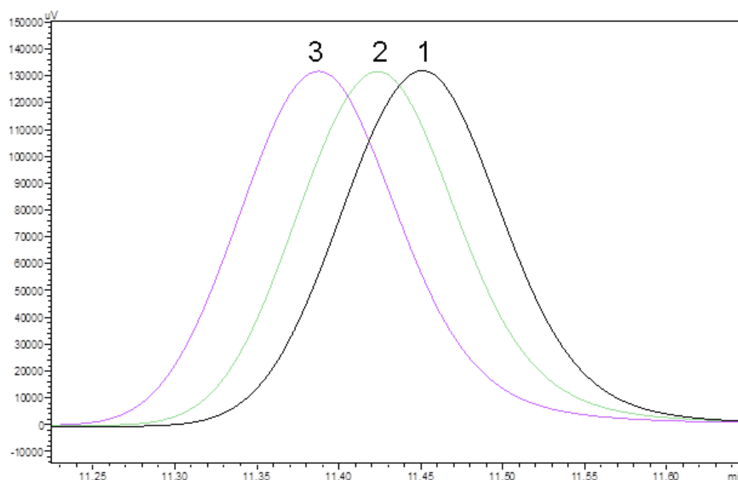


Figure 22. n-Octane peak in the chromatograms of the mixture 2 obtained using *in-house* made stainless steel column after 1 (1), 20 (2) and 40 (3) hours conditioning. Chromatographic conditions are described in Experimental.

Strongly active analytes normally give lower peak heights and responses because of their adsorption onto the column active sites. With the stationary phase loss the column active sites became more easily assessable and interact stronger with the active analytes. It is particularly evident for 1-hexanol peak that after conditioning became even broader and significantly lower (Fig. 23).

Peak height ratios between the tested compounds and an inert n-octane have been calculated after conditioning of the column for 1 hour and 40 hours. As can be seen from the results presented in Table 7, peak ratios for inactive benzene, cumene and slightly polar chlorobenzene were almost identical independently on the conditioning time. On the other hand, peak ratios of strongly active aniline and 1-hexanol significantly decreased after 40 hours conditioning. This can be attributed to the stationary phase loss and consequent deterioration of the column inertness with conditioning at high temperature.

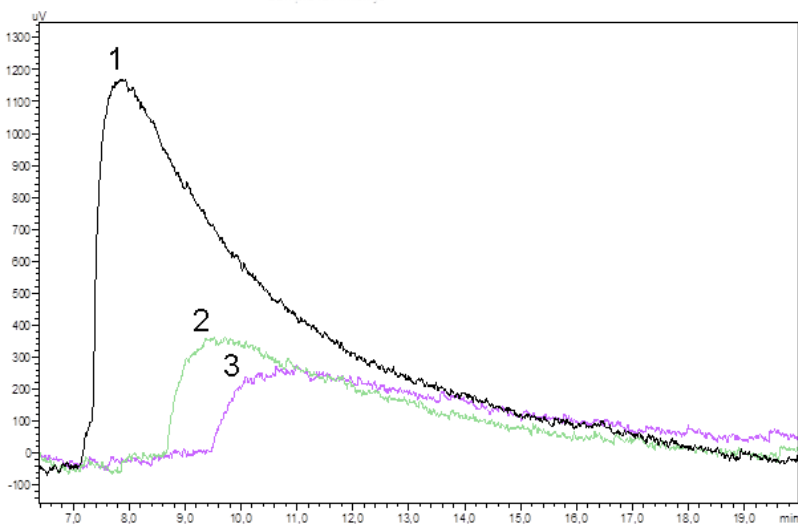


Figure 23. 1-Hexanol peak in the chromatograms of the mixture 3 obtained using *in-house* made stainless steel column after 1 (1), 20 (2) and 40 (3) hours conditioning. Chromatographic conditions are described in Experimental.

Table 9. Peak height ratios between test compounds and n-octane after conditioning of the *in-house* column at 200°C

Compound	1 hour	40 hours
Benzene	1.027	1.033
Chlorobenzene	1.022	1.040
Pentyl acetate	0.603	0.630
Cumene	0.808	0.818
Aniline	0.058	0.041
2-Butanone	0.849	0.769
1-Hexanol	0.005	0.001

4.1.5. Column application

Concerning above presented column evaluation it is evident that the column has significant drawbacks and would not fit properly for the analysis of the samples containing active analytes such as amines, alcohols, organic acids, aldehydes or ketones. On the other hand, the column could be applied for the determination of nonpolar and inactive compounds. The chromatogram presented in Fig. 24 demonstrates that alkanes could be successfully separated with good peak shapes and short analysis time.

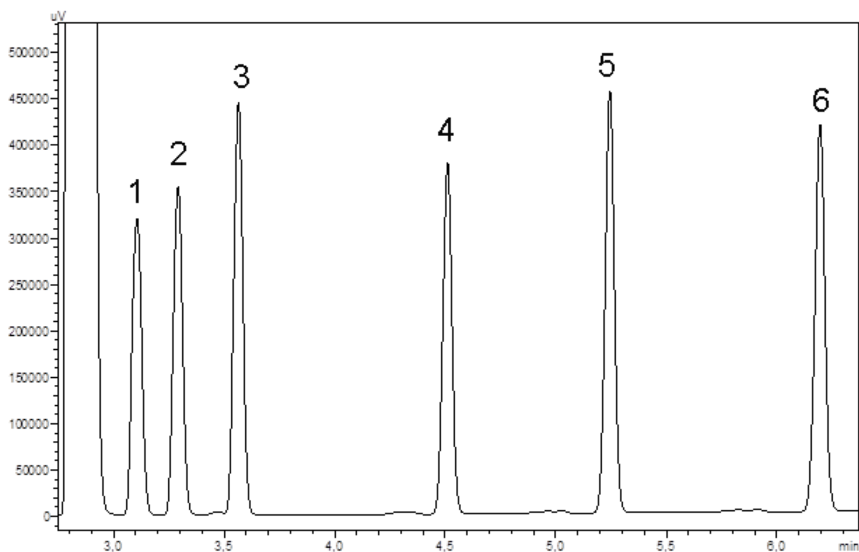


Figure 24. Chromatogram of the mixture 5 obtained using *in-house* made stainless steel column. 1 – n-octane, 2- n-nonane, 3 - n-decane, 4 - n-dodecane, 5 - n-tridecane, 6 - n-tetradecane. Chromatographic conditions are described in Experimental.

In conclusion of this part we could state that the stainless steel columns are probably the best choice for high-temperature chromatography. However, the metal surface with active sites is matter of great concern when dealing with active analytes. On the other hand, active analytes enable in a great extent to reveal the deficiencies of a column. The obtained results demonstrated poor column performance towards active analytes indicating the presence of active sites on the column surface. The column can be applied only for the analysis of mixtures containing nonpolar compounds. The further work should be directed towards deactivation of inner column walls and thus to the improvement of column's inertness. In order to enhance thermal stability and longevity of the column, bonded or crosslinked stationary phases should be used.

4.2. Determination of dyes and marker in diesel using high performance liquid chromatography

4.2.1. Selection of detection wavelengths

The sample analysis was made with PerkinElmer Series 200 (PerkinElmes Instruments, USA) equipped with UV/Vis detector. Using this type of detector before analysis need to know what wavelength to use for fuel dyes and marker to detect. Knowing the wavelength at which is the best analyte absorption we

can produce the best results on the chromatogram. It lets achieve the highest detection sensitivity of analytes. In order to find out the necessary wavelengths needed to carry out the spectrophotometric analysis of the analytes. Spectrophotometric analysis was prepared individual dyes and marker solutions with concentration was 5 mg / L. The spectrophotometric analysis of the results presented in Fig. 25.

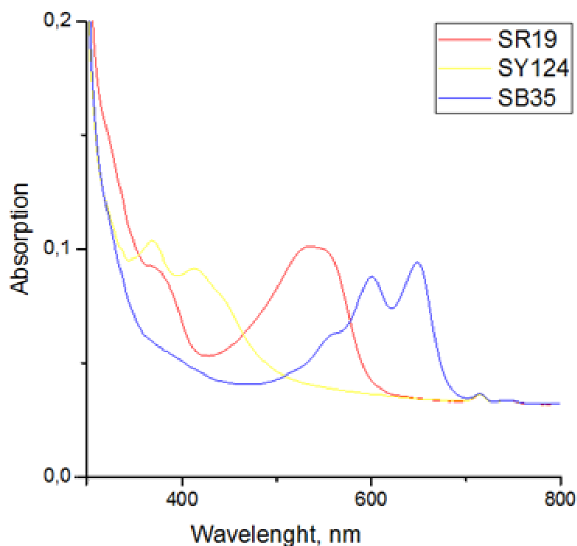


Figure 25. Fuel dyes and marker spectrophotometric analysis results, concentration of each analyte 5mg/L.

From the Fig. 25 we can see that the best dyes absorption is at the 535 nm wavelength for “Solvent Red – 19” and 650 nm wavelength for “Solvent Blue – 35” also for the marker “Solvent Yellow – 124” the best absorption is at the 390 nm wavelength.

4.2.2. Optimization of separation conditions

For the first dependence of fuel dyes and marker retention time from different eluent it was chosen the composition of hexane, toluene and MTBE. This kind of eluent composition was find in DIN – 51430, but this composition is appropriate just for “Solvent Red – 19” and “Solvent Yellow – 124” there is no results for “Solvent Blue – 35”. So the gradient composition of moving phase is till the 18 min of analysis moving phase consist of hexane/toluene (75/25 vol. %) after that for 5 more min into moving phase come MTBE (8 vol. %) to eluate most polar compounds. During this type of analysis, the retention time between analytes is very big, and if you increase the MTBE

concentration in moving phase the repeatability decrees and there must be longer period of HPLC column conditioning.

For further fuel dyes and marker retention time dependence analysis as a moving phase was chosen toluene and ethyl acetate. This kind of moving phase was found in article [142]. In this article was written how to detect "Solvent Blue – 35" and marker "Solvent Yellow – 124", but nothing was written about "Solvent Red – 19" detection. The analysis was made with this kind of moving phase which consist of toluene/ethyl acetate (98/2 vol. %) without gradient. The flow of moving phase 1 mL/min. After few analyses it was seen that "Solvent Red – 19" has no retention and go out with unrestrained phase retention time. Increasing or decreasing ethyl acetate concentration has no effect on "Solvent Red – 19" retention time. Also there was large retention time difference between "Solvent Blue – 35" and "Solvent Yellow – 124".
After both type of analysis methods it seems that hexane helps to retain "Solvent Red – 19" and ethyl acetate decrease "Solvent Blue – 35" and "Solvent Yellow – 124" sorption on stationary phase. So we came up with idea that hexane/toluene/ethyl acetate mixture as a moving phase must help to get best results. First we tried moving phase consisting from hexane/toluene/ethyl acetate (44,5/44,5/1 vol. %). The flow was 1 mL/min. This kind of analysis were made ant it shows that "Solvent Red – 19" retention time increased. But the problem was that "Solvent Blue – 35" and "Solvent Yellow – 124" retention times increases too. All retention times with different eluent is shown in Fig. 26. So for the further analysis it was chosen the last eluent composition, because the composition of eluent can be optimized and the gradient can be applied for the best performances.

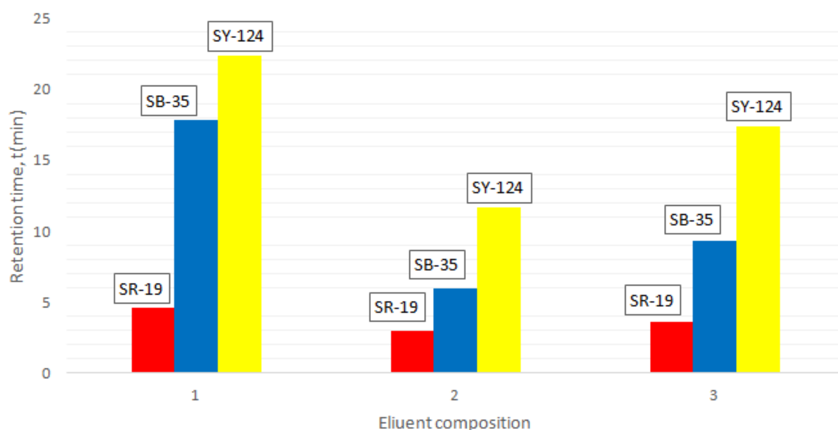


Figure 26. Retention time from different eluent. 1 – hexane/toluene/MTBE (75%/25%/8%); 2 – toluene/ethyl acetate (98%/2%); 3 – hexane/toluene/ethyl acetate (44,5%/44,5%/1%).

4.2.3. Determination of method parameters

Adopted and optimized moving phase composition for the analysis is in Table 10.

Table 10. Moving phase composition for the analysis

Step	Time (min)	Flow rate (mL/min)	Hexane (%)	Toluene (%)	Ethyl acetate (%)
Conditioning	7.0	1.0	30	70	0
1	2.0	1.0	30	70	0
2	8.0	1.0	0	93	7
3	5.0	1.0	0	93	7

Various analysis of fuel dyes and marker showed, that for best retention and separation of analytes is when three different eluents are used: hexane, toluene and ethyl acetate. Using gradient composition of these three eluents the best results could be reached. Moreover, there was made a que of analysis to find out the shortest time for conditioning the separation column to eliminate ethyl acetate from it. So the conditioning of separation column before every analysis took 7 minutes. Total time of analysis is 22 minutes. Retention times of analytes is shown at Fig. 27. After eluent optimization follows calibration curve and real samples analysis.

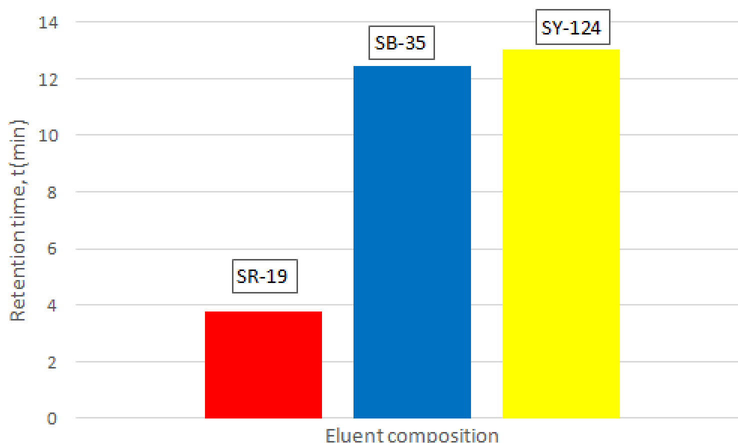


Figure 27. Fuel dyes and marker retention times.

4.2.4. Limit of detection (LOD) and limit of quantification (LOQ)

First characteristic of all chromatographic systems is column effectiveness which consist of: theoretical plates number (N) and its height (H).

$$N = 5{,}54 \times \left(\frac{3{,}75}{(3{,}827 - 3{,}702)}\right)^2 = 4986$$

$$H = \frac{4984}{250} = 19{,}94\ (mm)$$

For example, in the study [148] after optimization the method was validated in-house by analyzing several parameters (linearity, limit of detection LOD, limit of quantification LOQ, robustness, recovery, precision, and accuracy) to determine its effectiveness. Linearity was measured in the 2.5-50 mg/L range; furthermore, intra-day and inter-day precision values were lower than 15%, while the LOD and LOQ were lower than 1 and 1.5 mg/kg, respectively, for all compounds considered. It was concluded, that the main advantages of this revised protocol were: (i) significant reduction in time and solvents needed for each analytical determination; (ii) application of HPLC as an alternative to traditional LC, carried with manually packed glass columns, thus simplifying the separation step. It was also demonstrated that volume overload is a critical limitation in Reversed Phase (RP)-HPLC purification of pharmaceutical compounds [149].

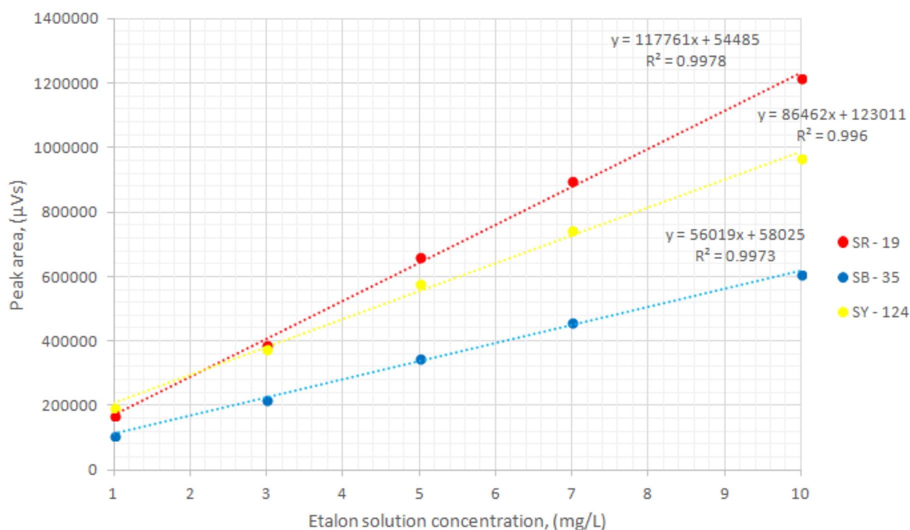


Figure 28. Fuel dyes and marker calibration curve. Peak area dependence from etalon solution concentration.

In our investigations all the calculations were made from etalon solution with concentration - 5 mg/L. After eluent optimization and necessary equations calculation there were made five etalon solutions with concentration various from 1 mg/L to 10 mg/L. All calibration curves results are shown in Fig. 28 and Table 11.

Table 11. The main characteristics of analysis

Analyte	t_R, min	Line equation	r^2	LOD, mg/L	LOQ, mg/L	SSN*, %
SR – 19	3.75	y = 117761x - 54485	0.998	0.56	1.88	2.1
SB – 35	12.04	y = 86462x - 123011	0.997	0.63	2.10	5.0
SY - 124	12.44	y = 56019x - 58025	0.996	0.77	2.55	1.0

4.2.5. Analysis of diesel samples

Optimized method was successfully adopted for real samples. The samples were received from the firm which engaged fuel dying and marking. Two types of fuel with low taxes were analyzed. First was red color fuel which dyed with "Solvent Red – 19" and marked with "Solvent Yellow – 124". Second was green color fuel which dyed "Solvent Blue – 35" and marked "Solvent Yellow – 124". The analysis results are in Table 12.

Table 12. Real samples analysis results

Sample	Dye	Indicated c (mg/L)	Necessary c*, mg/L	Marker	Indicated c(mg/L)	Necessary c*(mg/L)
Green color diesel fuel	SB – 35	0.61	5.0	SY – 124	4.42	8.5 – 12.7
Red color diesel fuel	SR - 19	0.45	6.0	SY - 124	4.63	8.5 – 12.7

*- ***necessary concentration,*** *the lowest concentration of necessary dye or marker in fuel, that determined in LR Ministry of Energy [1].*

CONCLUSIONS

1. In the presented work stainless steel capillary column for gas chromatography was developed. For the evaluation of an *in-house* prepared stainless steel column coated with PDMS stationary phase test probes have been suggested. It was also demonstrated that the commercial *Elite-1* column was more efficient with the minimal plate height of 0.295 mm, meanwhile for the prepared column in this study the minimal plate height was 0.386 mm.
2. Column were suitable fr elution of non polar components like benzene, n-octane, cumene and chlorbenzene
3. The inertness of the column is insufficient to elute compounds of various polarities having a strongly expressed dipole moment, basic or acidic properties.
4. Column demostrated moderate termal stability. After 40 hours of conditioning 200 °C the shift of retention time of n-octane has been less than 0,5 %.
5. The further work should be directed towards deactivation of inner column walls and thus to the improvement of column's inertness. In order to enhance thermal stability and longevity of the column bonded or crosslinked stationary phases should be used.
6. Column properties are enough to use it in future tests as column-thermostat.
7. Determination of dyes and marker in diesel using high performance liquid chromatography was optimized in this PhD work. It was determined that the best dyes absorption was at the 535 nm wavelength for "Solvent Red – 19" and 650 nm wavelength for "Solvent Blue – 35". The best absorption was determined at the 390 nm wavelength also for the marker "Solvent Yellow – 124".
8. The developed method was applied for the analysis of real samples of dyed diesel fuel. The method was able to detect the analytes that we were analizing in dyed diesel. Using this method concentration of 0.45 mg/L of SR-19 and 4.63 mg/L of SY-124 were determined in red dyed diesel, 0,61 mg/L of SB-35 and 4.42 mg/L of SY-124 in green dyed diesel sample.
9. It was clearly demonstrated that this developed HPLC method is capable to detect two dyes SR-19, SB-35 and one marker SY-124 in one injection; also it might be possible to adopt this method for other dyes and markers in similar systems.

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SUMMARY

Dėl savo paprastumo, jautrumo ir efektyvumo dujų chromatografija yra vienas iš svarbiausių analizės įrankių analizinės chemijos srityje. Ši analizės rušis plačiai taikoma kiekybinėje ir kokybinėje organinių junginių analizėje, o taip pat ir šių junginių gryninime. Daugybė reikalingų tyrimų medicinoje, aplinkos apsaugoje, maisto, naftos pramonėje ir begalėje kitų sričių yra atliekama dujų chromatografija.

Dujų chromatografinės kolonėlės efektyvumui įvertinti yra naudojami kokybės kontrolės standartai, kurie leidžia įvertinti kolonėlės deaktyvavymo kokybę, stacionarios fazės kokybę ir kolonėlės atitikimą analogiškai kolonėlei. Kolonėlės kokybei įvertinti naudojamos įvairių klasių medžiagos, leidžiančios atskleisti kolonėlės savybes ir tinkamumą pasirinktų medžiagų analizei. Labai svarbi chromatografinės analizės sritis yra skystinė chromatografija. Jei dujinė chromatografija yra naudojama lakių organinių junginių analizei, tai skyčių chromatografija naudoma analizuoti junginius ar ištisas junginių klases, kurie nėra pakankamai lakūs arba termiškai stabilūs, kad būtų galima atlikti jų analizę dujų chromatografijos metodu.

Viena iš skyčių chromatografijos pritaikymo sričių yra dažiklių analizė. Daugelyje pasaulio valstybių skirtingoms iškastinių angliavandenių kuro rūšims ir jų panaudojimo sritims taikoma skirtinga apmokestinimo politika. Kad būtų lengviau suvaldyti skirtingais tarifais apmokestinto kuro judėjimą ir naudojimą pagal tikslinę paskirtį yra naudojmi kuro dažikliai. Šie dažikliai suteikia kurui specifinę spalvą ir leidžia lengvai identifikuoti kuro paskirtį. Lengvatinio apmokestinimo dažytas kuras yra naudojamas žemės ūkyje, pastatų šildymui, komercinėje žvejyboje. Skirtingų dažiklių panaudojimas leidžia diferencijuoti kuro panaudojimo sritis. Kuro dažymas atliekamas pagal specifinę metodiką. Tačiau universalaus metodo, kuris leistų vienos analizės metu nustatyti dažų rūšį ir koncentraciją, kol kas nėra. Skirtingiems dažikliams nustatyti naudojami įvairus spektrometriniai, elektrocheminiai, dujų ar skysčių chromatografijos metodai.

Disertacijos naujumas

Disertacija sudaryta iš dviejų dalių. Pirmoje disertacijos dalyje pateikiami duomenys apie atlikrus kuriant nerudijančio plieno karkasą turinčia kolonėlę.

Didelė dauguma dujų chromatografijos darbų atliekama naudojant termostato, kuriame patalpinta analitinė kolonėlė, temperatūrinį gradientą. Temperatūrinio gradiento kitimo greitis ir tikslumas yra esminis faktorius kaip galima valdyti analyčių judėjimo per kolonėlę. Nuo pat dujų chromatografijos

kaip metodo sukurimo analitinė kolonėlė buvo talpinama į uždarą konteinarį arba kitaip termpostatą kuriame sumontuotas kaitinimo elementas, temperaturos jutiklis, ventiliatorius ir kiti komponentai skirti palaikyti norima temperatūrą ir užtikrinti jos kitimo stabilumą. Visi šie įrenginiai sudaro ganėtinai didelę struktūrą kurios šiluminė talpa yra gana didelė. Didelė šiluminė talpa sąlygoja ir didelę inerciją, tai lemia kad temperatūros kitimo greitis apsiriboja iki keliasdešimt laispnių per minutę.

Taip pat, vystantis dujų chromatografijai buvo prieita prie visuotinai priimto standarto, kad geriausia medžiaga kolonėlės karkasui yra cilindras suformuotas iš išlydytas cilicio dioksido (angl. fused silica), nes jis pasižymi dideliu interiškumu visoms dujų chromatografijoje naudojamoms medžiagoms.

Mes iškėlėme mintį kad pagaminus kolonėlės karkasą iš elektrai laidžios medžiagos būtų galima prie jos prijungti elektros įtampą ir laidumo elektrai sąvybę panaudoti temperatūros gradiento formavimui. Tai yra kolonėlės karkasas būtų tuo pačiu ir termostatas.

Antroje disertacijos dalyje pateikiama medžiaga apie analitines metodikos skirtos identifikuoti kuro dažiklius kure parengimą

Europos sąjungoje remiant žemės ūkio veiklą yra taikomi lengvatiniai mokesčiai kurui naudojamam žemės ūkio veikloje. Siekinat užkardyti neteisėtą lengvatinio apmokestinimo kuro panaudojimą, šis kuras yra žymimas specialiais dažais pagal kurių spalvą ir kiekį galima būtų įvertinti neteistos veiklos mastą. Kiekviena šalis narė individualiai sprendžia kokius dažiklius panaudoti. Lietuvoje yra naudojamos trys medžiagos „Solvent Blue – 35“ „Solvent Red – 19“ „Solvent Yellow – 124“. Nors yra rekomendacinės metodikos kaip atlikti kiekvienos medžiagos kiekybinius ir kokybinius tyrimus, nebuvo vieningos metodikos kuri leistų nustatyti visas medžiagas vienos analzės metu.

Mes parengėme metodika kurti leidžia nustatyti visas tris medžiagas taikant vienos injekcijos HPLC metodą.

Disertacijos tikslas

Šios disertacijos tikslas buvo pagaminti analitinę kolonėlę elektrai laidžiu karkasu ir sukurti bei validuoti chromatografinę metodiką kuro dažiklių analizei taip praplečiant dujų ir skysčių chromatografijos pritaikymo ir panaudojimo sritis. Disertacijos tikslams pasiekti buvo suformuluotos šios užduotys:

1. Pagaminti efektyvią nerūdijančio plieno kapiliarinę kolonėlę su stacionariąja metilpolisiloksano faze.

2. Ištirti ir įvertinti pasigamintos kolonėlės savybes testuojant skirtingomis medžiagomis, pasižyminčiomis skirtingomis savybėmis ir palyginti su komerciškai parduodamų lydyto silicio pagrindu pagamintų kolonėlių savybėmis.
3. Sukurti ir optimizuoti HPLC universalią metodiką LR ir ES naudojamų kuro dažiklių ir žymeklių analizei.
4. Patvirtinti sukurtą HPLC metodą jautriam dažiklių ir žymeklio koncentracijos degaluose (SR 19 ir SB 35; ir žymeklis: SY 124) aptikimui ir tiksliam nustatymui.

Eksperimentinė dalis

Kapiliarinės kolonėlės paruošimas:

Nerūdijančio plieno kolonėlei pagaminti iš vietinio tiekėjo buvo įsigytas 304 klasės 0,32 mm ID ir 0,5 mm OD nerūdijančio plieno kapiliaras. 30 m ilgio kapiliaro galas buvo suvyniojamas ant pasiruošto 17 cm diametro laikiklio.

Pirminis kapiliaro plovimas:

Paruoštas kolonėlės karkasas (suvyniotas ant laikiklio kapiliaras) buvo prijungiamas prie Perkin Elmer S200 skystinio chromatografo pompos ir plaunamas heksanu 30 min esant 0,5ml/min heksano srautui, kad būtų pašalintos nuo kapiliaro gamybos likusios organinės medžiagos (tepalai). Po plovimo heksanu kapiliaras tokiu pačiu principu buvo praplaunamas acetonu, isopropilo alkoholiu ir dejonizuotu vandeniu.

Išplovus kapiliarą nuo likutinių organinių tirpiklių, nerūdijantis plienas buvo pasyvuojamas. Kolonėlė buvo patalpinama į 60 °C termostatą, ir per kolonėlę 12 valandų 0,01 ml/min greičiu buvo pumpuojamas 20% azoto rūgšties tirpalas. Po to kolonėlė vėl buvo išplaunama dejonizuotu vandeniu, kol pH vertė pasiekdavo 7.

Stacionarios fazės dengimo ant nerūdijančio plieno kapiliaro technikos:

Nerūdijančio plieno kapiliarinės kolonėlės padengimui buvo naudojamos dvi technikos: dinaminė ir statinė. Dinaminio dengimo metu 5 ml šviežiai paruošto PDMS dangos tirpalo buvo pritraukiama į laboratorinį švirkštą. Švirkštas per adapterį prijungiamas prie išplauto ir išdžiovinto kapiliaro. Švirkštas fiksuojamas pastovaus greičio slenkančio judesio pompoje, kuri nustatytu 0,1 ml/min greičiu spausdavo švirkšto kojelę. Kai kapiliaras buvo užpildomas dengimo tirpalu, švirkštas buvo pritraukiamas oro ir prijungiamas

atgal prie kapiliaro. Tuo pačiu 0,1 ml/min greičiu stumiamas oras išstumdavo dengimo tirpalo perteklių. Tik mažas kiekis dengimo tirpalo likdavo ant kapiliaro vidinių sienelių. Likęs PDMS sluoksnio storis ant sienelės priklausė nuo dengimo tirpale buvusio PDMS kiekio. Po to, kai dengimo tirpalo perteklius buvo pašalintas iš kapiliaro, kapiliaras buvo prijungtas prie dujų chromatografo injektoriaus, ir buvo nustatytas 0,8 barg nešančiųjų dujų slėgis, nešančiųjų dujų tekėjimas per kolonėlę buvo paliekamas per naktį esant aplinkos temperatūrai, taip leidžiant išgarinti likusį tirpiklį. Po pirminio džiovinimo kolonėlės antras galas buvo prijungiamas prie FID detektoriaus. Dujų chromatografo termostato temperatūros programa buvo nustatoma į kėlimo rėžimą nuo 35°C iki 330°C esant 1°C/min temperatūros kilimui. Termostato temperatūrai pasiekus maksimalią vertę sistema buvo paliekama toje būsenoje iki FID detektoriaus registruojamas signalas nusistovėdavo ties pastovia verte.

Statinio dengimo metu, kapiliaras buvo užpildomas dengimo tirpalu tokiu pačiu principu kaip ir dinaminio dengimo atveju. Užpildyto kapiliaro galai buvo prijungiami prie T formos jungties, prie trečio T formos jungties galo buvo prijungiamas kitas kapiliaras, sujungtas su vakuumine pompa. Vakuuminės pompos pagalba buvo sukuriamas absoliutus 0,4 bar vakuumas. Užpildytas dengimo tirpalu ir prijungtas prie vakuuminės pompos kapiliaras buvo patalpinamas į termostatą. Termostato temperatūra buvo nustatoma artima tirpiklio, kuriame ištirpintas PDMS, virimo temperatūrai. Kapiliaro vakuumavimas buvo atliekamas, kol visas tirpiklis buvo išgarinamas. Dažniausiai tai užtrukdavo nuo 6 iki 12 valandų. Po to kai tirpiklis buvo išgarinamas kapiliaras buvo prijungiamas prie dujų chromatografo injektoriaus ir FID detektoriaus. Kolonėlės galutinis džiovinimas ir kondicionavimas buvo atliekamas taip pat kaip ir dinaminio dengimo atveju.

PDMS dengimo tirpalo paruošimas:

PDMS tirpalas dinaminiam dengimui buvo ruošiamas praskiedžiant pradinį PDMS reagentą heksanu santykiu nuo 1:4 iki 1:10 pagal masę. Tirpalas buvo ruošiamas šviežiai prieš kiekvieną dengimą. Vienu metu buvo paruošiama 5 g tirpalo. Paruoštas tirpalas buvo įdedamas į ultragarsinę vonelę 15 min, kad PDMS tolygiai pasklistų visame tūryje.

PDMS tirpalas statiniam dengimui buvo ruošiamas įdedant labai tiksliai pasvertą PDMS kiekį pentane. Reikiamas tirpalo kiekis buvo apskaičiuojamas darant keletą prielaidų:

a) kapiliaro vidinis paviršius yra idealus cilindras;

b) PDMS negaruoja ir nėra ištraukiamas vakuumavimo metu kartu su tirpikliu;

c) PDMS sluoksnis tolygiai nusėda ant viso kapiliaro vidinio paviršiaus.

Atskaitos tašku buvo pasirinktas 0,25 μm storio sluoksnis. Reikiamas PDMS kiekis apskaičiuojamas atimant iš kapiliaro vidinio tūrio be dangos kapiliaro vidinį tūrį su danga. Gautas skirtumas padalinamas iš PDMS tankio.

Mėginio paruošimas kuro dažiklių analizei:

Kuro ir degalų dažikliai („Solvent Blue – 35“ ir „Solvent Red – 19“) bei žymiklis („Solvent Yellow – 124“) buvo pasveriami ir tirpinami HPLC grynumo toluene. Skiedžiant gautus tirpalus buvo gauti mažesnės koncentracijos tirpalai, reikalingi atlikti analizėms bei kalibracinei kreivei nustatyti. Tirpalai taip pat buvo skiedžiami HPLC grynumo toluenu. Etaloninių tirpalų ruošimas ir skiedimas yra pateikta 1-oje lentelėje:

1 lentelė. Etaloninių tirpalų ruošimas ir skiedimas.

Naudojamas tirpiklis:		**Tankis (g/ml):**		
Toluenas		0,867		
Pirminių tirpalų paruošimas:				
Dažikliai	**m kolbos (g)**	**m dažiklių (g)**	**m tolueno (g)**	**V tolueno (ml)**
„SR – 19“	14,57572	0,00136	8,70252	10,0375
„SY – 124“	14,76953	0,01163	8,65990	9,9884
„SB – 35“	14,38766	0,00265	8,66853	9,9983
„Standartiniai tirpalai v1“				
Dažikliai	**c (mg/L)**		**c (mg/mL)**	
„SR – 19“	135,0229		0,1350	
„SY – 124“	1160,3275		1,1603	
„SB – 35“	264,1278		0,2641	

Iš „standartiniai tirpalai v1“ ruošiamas tarpinis standartinis dažiklių („Solvent Blue – 35“ ir „Solvent Red – 19“) ir žymiklio („Solvent Yellow – 124“) mišinys, kuriame dažiklių ir žymiklio koncentracijos yra po 20 mg/L. Ši koncentracija pasirinkta dėl to, kad ją skiedžiant bus lengvai gauti reikalingų koncentracijų tirpalai kalibracinei kreivei nustatyti. Dažiklių ir

žymiklio mišinio kalibraciniai tirpalai kalibracinei kreivei buvo ruošiami praskiedžiant tiksliai pasvertą tarpinio tirpalo tirpalo kiekį iki 2 ml.

Kalibracinių tirpalų paruošimo duoemnys pateikiami 2-oje lentelėje:

2 lentelė. Dažiklių ir žymiklio mišinio paruošimo kalibracinei kreivei sąlygos

Dažiklis	**Kal. St.**	**Dažiklio Konc. (mg/L)**	**Tarpinio tirpalo tūris (L)**	**Tarpinio tirpalo (g)**	**Tolueno masė (g)**
„SR – 19“	1	1	0,0001	0,0867	1,6473
„SB – 35“	1	1	0,0001	0,0867	1,6473
„SY – 124“	1	1	0,0001	0,0867	1,6473
„SR – 19“	2	3	0,0003	0,2601	1,4739
„SB – 35“	2	3	0,0003	0,2601	1,4739
„SY – 124“	2	3	0,0003	0,2601	1,4739
„SR – 19“	3	5	0,0005	0,4335	1,3005
„SB – 35“	3	5	0,0005	0,4335	1,3005
„SY – 124“	3	5	0,0005	0,4335	1,3005
„SR – 19“	4	7	0,0007	0,6069	1,1271
„SB – 35“	4	7	0,0007	0,6069	1,1271
„SY – 124“	4	7	0,0007	0,6069	1,1271
„SR – 19“	5	10	0,0010	0,8670	0,867
„SB – 35“	5	10	0,0010	0,8670	0,867
„SY – 124“	5	10	0,0010	0,8670	0,867

Tirpalų kalibracinei kreivei koncentracijos patikrinimas					
Kalibracinės taškas	1	2	3	4	5
Standarto konc. (mg/L)	1	3	5	7	10
Mėgintuvėlio svoris (g)	2,7799	2,7520	2,7392	2,7174	2,7499
Masė su standartu (g)	2,8667	3,0201	3,1800	3,3164	3,6190
Masė su toluenu (g)	4,5140	4,4841	4,4693	4,4428	4,4950
Standarto masė (g)	0,0869	0,2681	0,4408	0,5990	0,8691
Tolueno masė (g)	1,1647	1,4641	1,2837	1,1264	0,8760
Masė standarto ir tirpiklio (g)	1,7341	1,7321	1,7245	1,7254	1,7451
Visas tūris (mL)	2,01	2,00	2,00	2,00	2,02
Standarto konc. (mg/L)	1,00	3,10	5,11	6,94	9,96

Normalių fazių skysčių chromatografinėms analizėms atlikti kaip judri fazė buvo naudojami šie tirpikliai: toluenas, heksanas, etilacetatas, metiltretbutileteris.

Realus mėginys t.y. kuro ir (ar) degalų mėginys, kuriame yra dažiklio ir žymiklio, prieš analizę buvo filtruojamas pro 0,45 μm (filtro porų dydis) hromafil Xtra PTFE – 45/25“ (Macherey – Nagel, Vokietija) filtrą. Filtro skersmuo - 25 mm. Filtras yra pagamintas iš teflono (PTFE – *angl. polytetrafluoroethylene*). Prafiltruotas mėginys patalpinamas į 2 ml chromatografinį indelį.

Chromatografinių tyrimų sąlygos

Dujų chromatografinės analizės sąlygos

Dujų chromatografinė analizė buvo atliekama naudojant Shimadzu GC-2010 Plus dujų chromatografą su integruota AOC-20i automatine mėginių įvedimo sistema split/splitless injektoriumi ir liepsnos jonizacijos detektoriumi. Analizėms atlikti buvo naudojama laboratorijoje pagaminta nerūdijančio plieno 30 m ilgio ir 0,32 mm skersmens ID kolonėlė, padengta 1 mm PDMS sluoksniu, ir Perkin Elmer kompanijos lydyto kvarco kolonėlė Elite-1 (100% PDMS). Helis buvo naudojamas kaip nešančios dujos, nešančiųjų dujų srautas buvo palaikomas 1,2 ml min^{-1}. Injektoriaus temperatūra buvo palaikoma 250 °C, injekcija buvo atliekama su srauto padalijimo rėžimu 10:1. Liepsnos jonizacijos detektoriaus temperatūra buvo palaikoma 250 °C. Pagalbinis srautas detektoriuje buvo 30 ml min^{-1}, pagalbinio srauto dujos – helis. Degimo dujos vandenilis - 40 ml min^{-1}, degimo palaikymo dujos sausas oras - 400 ml min^{-1}. Chromatografo termostato programa: 1) analizuojamiems tirpalams 1, 2 ir 6 termostato temperatūra buvo palaikoma izokratinė 100 °C; 2) analizuojamiems tirpalams 3 ir 4 termostato temperatūra buvo palaikoma izokratinė 60 °C; 3) analizuojamam tirpalui 5 buvo naudojama programuojamos temperatūros rėžimas 170 °C 1 min, nuo 170 iki 200 °C naudojamas 7 °C min^{-1} temperatūros kėlimas ir galiausiai palaikoma 3 min esant 200 °C.

HPLC analizės parametrai

Chromatografinė analizė buvo atliekama naudojant Perkin Elmer S200 (PerkinElmer Instruments, USA) skystinį chromatografą su UV/VIS detektoriumi. 200 μL tūrio injekcijos kilpa buvo naudojama mėginio įvedimui. Analizėms atlikti buvo naudojama Zorbax Rx-SIL kolonėlė (250

mm × 4,60 mm ID, granulių dydis 5 µm) (Agilent Technologies, USA), tirpiklių maišytuvas 150 µL (PerkinElmer Instruments, USA). Realūs mėginiai išfiltruoti per 0,45 µm PTFE ir supilti į 2 ml mėgintuvėlius. Skystinio chromatografo mobilios fazės srautas buvo nustatytas 1 ml/min.

Rezultatų aptarimas

Laboratorijoje pagamintos nerūdijančio plieno kolonėlės dujų chromatografijai savybių tyrimas

Mobilios fazės srauto optimizavimas

Kolonėlės efektyvumas priklauso nuo mobilios fazės srauto. Esant optimaliam srautui teorinės lėkštelės aukštis yra mažiausias. Todėl pirmiausiai buvo optimizuotas mobilios fazės srautas pro pačių pasigamintą kolonėlę ir komercinę PerkinElmer *Elite-1*. Optimaliam srautui nustatyti buvo naudojamas standartinis n-dekano tirpalas dichlormetane. Helio (nešančiųjų dujų) srautas buvo keičiamas nuo 0,5 iki 7 ml min^{-1}. Teorinės lėkštelės aukštis buvo apskaičiuojamas pasinaudojant formulėmis 1 ir 2:

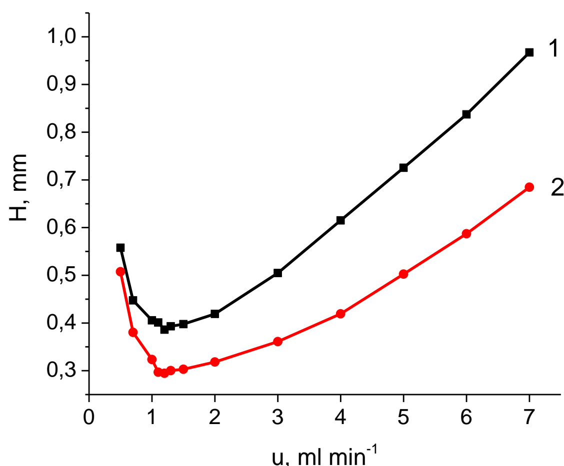
$$N = \left(\frac{t_r}{\frac{w_h}{2.35}}\right)^2 = \left(\frac{2.35 t_r}{w_h}\right)^2 = 5.54\left(\frac{t_r}{w_h}\right)^2 \quad (1)$$

ir

$$H = \frac{L}{N} \quad (2)$$

Kur N- teorinių lėkštelių skaičius; $\boldsymbol{t_R}$ – n-dekano sulaikymo laikas; $\boldsymbol{w_{0.5}}$ – n-dekano smailės plotis ties 0,5 smailės aukščio, L – kolonėlės ilgis.

Kaip galima matyti iš 1 paveikslo, tiek laboratorijoje pagamintos, tiek komercinės kolonėlės optimalus mobilios fazės greitis yra 1,2 ml min^{-1}. Iš šio rezultato taip pat matoma, kad komercinė Elite-1 kolonėlė yra efektyvesnė esant minimaliam teorinės lėkštelės aukščiui. Elite-1 minimalus teorinės lėkštelės aukštis 0,295 mm, tuo tarpu laboratorijoje pagamintos kolonėlės minimalus teorinės lėkštelės aukštis 0,386 mm. Abiejų kolonėlėlių danga yra PDMS, abiejuose tyrimuose buvo naudotos tos pačios helio dujos.



1 pav. Mobilios fazės srauto įtaka teorinės lėkštelės aukščiui *laboratorijoje pagamintai* (1) ir *Elite-1* (2) kolonėlėms.

Elite 1 kolonėlės vidinis diametras yra 0,25 mm, o laboratorijoje pasigamintos kolonėlės - 0,32 mm. Tai gali būti viena iš priežasčių, kodėl Elite 1 kolonėlės efektyvumas yra geresnis. Kitas galimas paaiškinimas, kad laboratorijoje pasigamintos kolonėlės dangos tolygumas nėra toks vienodas kaip komercinės kolonėlės.

Testavimo medžiagų parinkimas

Kolonėlės inertiškumui nustatyti matuojamas, matavimams parinktų medžiagų smailių asimetriškumas. Idealiu atveju eliuojant medžiagą per kolonėlę gaunama simetriška, apversto varpo formos smailė. Medžiagai turint papildomą, specifinę saveiką su kolonėle stebimas šios smailės asimetriškumas, kuris apriboja kolonėlės pritaikymo galimybes. Asimetriškumas gali pasireikšti kaip smailės galinės dalies ištempimas, sumažėjimas ar visiškas išnykimas iš chromatogramos. Siekiant įvairiapusiškai įvertinti kolonėlės inertiškumą svarbu parinkti tokius junginius, kurie leistų įvertinti kolonėlės terminį stabilumą, karkaso sąveikos su junginiais laipsnį, taip pat stacionarios fazės storį. Netinkamai parinkti testavimo junginiai gali užmaskuoti kolonėlės efektyvumo trūkumus, kaip kad junginiai, neturintys savybių specifiškai sąveikauti su kolonėlės karkasu, gali neparodyti kolonėlės karkaso pasyvavimo spragų. Idealūs testavimo junginiai turi būti mažos molekulinės masės, turintys žemą virimo temperatūrą ir neturintys erdviškai užstotų funkcinių grupių. Tokių junginių sąveika su

stacionaria faze ir kolonėlės karkasu yra maksimaliai išreikšta ir matoma chromatogramoje.

GC kolonėlės tyrimams yra komerciškai prieinamas Grob'o testinis mišinys. Tačiau šiame mišinyje esantys junginiai, mūsų nuomone, nėra parinkti optimaliai. Kaip pavyzdys, šiame teste esantis 2,6-dimetilfenolis yra skirtas įvertinti kolonėlės baziškumą, arba 2,6-dimetilanilinas skirtas įvertinti kolonėlės rūgštingumą. Šiuose junginiuose funkcinės hidroksilo ir amino grupės yra erdviškai dengiamos šalia esančių metilo ir fenilo grupių ir neleidžia pilnai pasireikšti grupių galimai sąveikai.

Mes peržvelgėme daugiau nei 30 junginių, kurie būtų tinkamesni tiriant kolonėlės savybes ir atrinkome devynis junginius: benzeną; n- oktaną, chlorbenzeną, pentilacetatą, kumeną, aniliną, 2-butanoną, 1- heksanolį ir propano rūgštį.

Tam kad eliuuoti šiuos junginius per kolonėlę, iš vienos pusės išlaikant pakankamai žemą kolonėlės temperatūrą, tačiau labai neištęsiant eliuacijos laiko ir išvengiant komponentų smailių persidengimo, junginiai buvo suskirstyti į keturias grupes. Junginių grupės pateiktos 3-ioje lentelėje. 2,6-dimetilanilinas Grobo testavimo mišinyje buvo pakeistas anilinu, o 2,6-dimetilfenolis pakeistas propano rūgštimi. Anilino ir propano rūgšties molekulės neturi tokių erdvinių apribojimų kaip jų pirmtakai, todėl gali daug laisviau sąveikauti su kolonėlės stacionaria faze ir karkasu. Taip pat norėdami padaryti testavimo mišinį dar efektyvesniu, pakeitėme 1-oktanolį į 1-heksanolį. Alifatinės grandinės ilgio sutrumpėjimas sumažina molekulės nepolinės dalies ilgį, taip labiau išreikšdamas hidroksilo funkcinės grupės aktyvumą. Aukštesnėje temperatūroje testavimui naudojamų junginių sąveikos jėgos silpnėja ir padaro juos ne tokiais efektyviais. Dėl šios priežasties Grob'o testavimo mišinyje dekano, nondekano ir diodekano rūgščių metilo esterius pakeistas pentilacetatu.

3 lentelė. Kolonėlių testavimo junginių tirpalai dichlormetane

Mišinys	Junginiai (10 mg ml^{-1} kiekvieno)
1	benzenas, n-oktanas, chlorbenzenas, pentilacetatas, kumenas, anilinas
2	2-butanonas, n-oktanas
3	1-heksanolis, n-oktanas
4	propano rūgštis, n-oktanas

Kolonėlės inertiškumo vertinimas

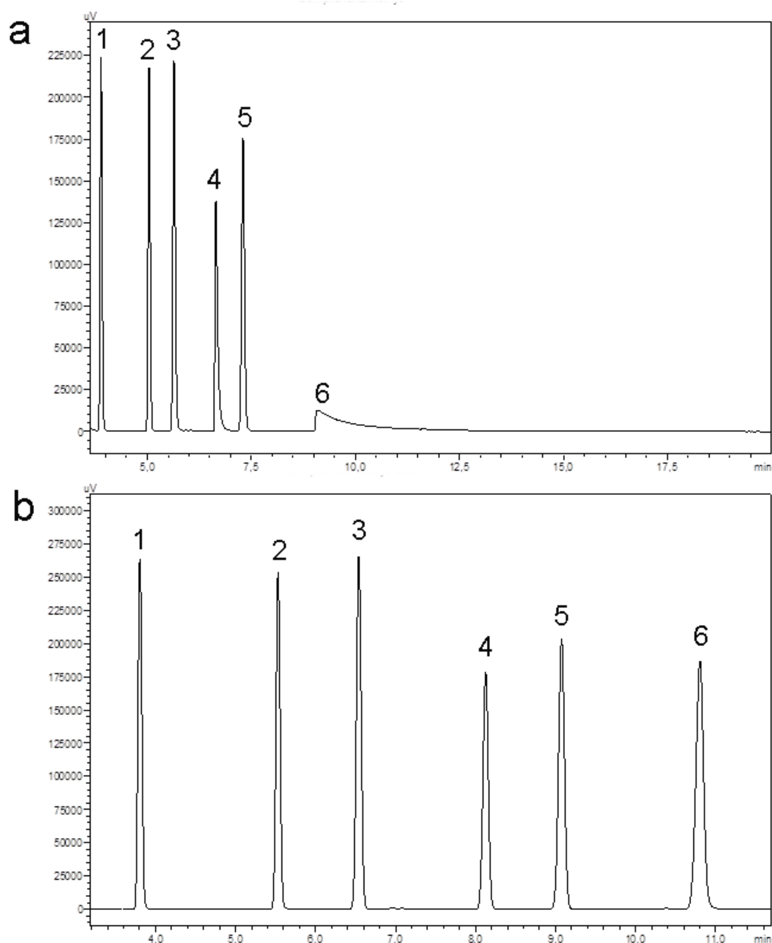
Kolonėlės inertiškumas buvo vertinimas atliekant chromatografines testinių mišinių analizes ir vertinant analičių smailių formas. Atlikus mišinio 1 chromatografinę analizę simetriškos smailės buvo stebimos tik nepoliniams junginiams (2a pav.). Benzeno, n-oktano, ir kumeno smailės buvo simetrinės ir palyginamos su tų pačių komponentų smailėmis, gautomis leidžiant mišinį 1 per komercinę Elite-1 kolonėlę (4 lentelė ir 2 pav.). Didėjant junginių poliškumui, smailių asimetriškumas didėjo nuo 1,17 silpnai poliškam chlorbenzenui iki 2,6 labiau poliškam pentilacetatui. Polinių junginių smailių dešinio krašto asimetriškumas – tempimas gali būti sąlygojamas dipolis-dipolis sąveikos tarp analitės ir kolonėlės karkaso medžiagos. Tokios molekulės kaip esteriai ar ketonai yra polinės ir todėl taip pat sąveikauja su kolonėlės karkasu, kas akivaizdžiai matosi iš 2-butanono smailės formos (3 pav.; 4 lentelė).

4 lentelė. Smailių asimetriškumas esant 10% smailės aukščio.

Junginys	*Laboratorijoje pagaminta kolonėlė*	*Elite-1*
Benzenas	1,10	1,05
n-Oktanas	1,06	1,02
Chlorbenzenas	1,17	1,01
Pentilacetatas	2,60	0,99
Kumenas Anilinas	1,08	0,97
2-Butanonas	11,0	0,94
1-Heksanolis	3,01	1,11
	11,1	0,99

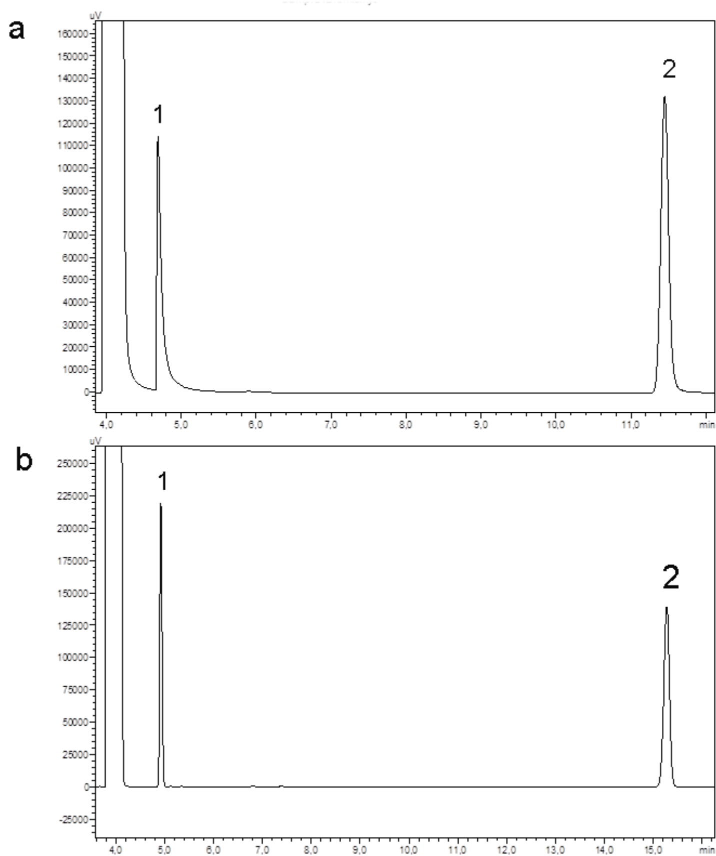
Labai didelis smailės tempimas buvo stebimas anilino smailei. Anilino smailės asimetriškumas, esant 10% smailės aukščiui, buvo 11. Anilinas yra

bazinė molekulė, todėl jos asimetriškumas indikuoja rūgštinių aktyvių centrų buvimą kolonėlėje. Anilinas gali formuoti vandenilinius ryšius, todėl jo smailės tempimas indikuoja galimą vandenilinį ryšį galinčių formuoti aktyvių centrų buvimą kolonėlėje.



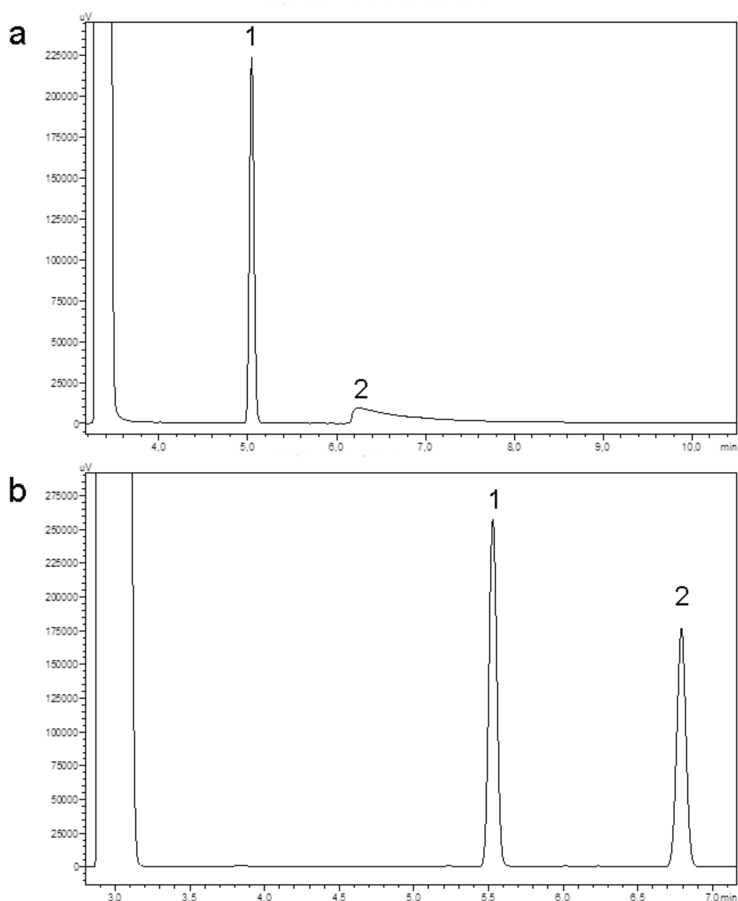
2 pav. Chromatogramos gautos analizuojant testinį mišinį 1, naudojant laboratorijoje pasigamintą kolonėlę (a) ir komercinę Elite-1 (b). 1 –benzenas; 2 – n-oktanas; 3 –chlorbenzenas; 4 – pentilacetatas; 5 – kumenas; 6 – anilinas. Chromatografinės sąlygos aprašytos ankstesniame skyriuje.

Labai asimetrinė 1-heksanolio smailė taip pat gali būti paaiškinima vandenilinio ryšio susidarymu tarp molekulės ir kolonėlės karkaso aktyviais centrais (3, 4 pav.; 4 lentelė). Propano rūgšties sąveika su kolonėle buvo ypatingai stipri ir jos smailė nebuvo fiksuojama laboratorijoje pasigamintoje kolonėlėje.



3 pav. Chromatogramos gautos analizuojant testinį mišinį 2, naudojant laboratorijoje pasigamintą (a) ir *Elite-1* (b) kolonėles. 1 – 2-butanonas, 2 – n-oktanas. Chromatografinės sąlygos aprašytos ankstesniame skyriuje.

Palyginimui, anksčiau minėtų polinių komponentų smailės gautos analizuojant analites komercine Elite 1 kolonėle, esant toms pačioms chromatografinėms sąlygoms, buvo simetriškos (žiūrėti Paveiklai 2b, 3b ir 4b bei lenetelė 4).



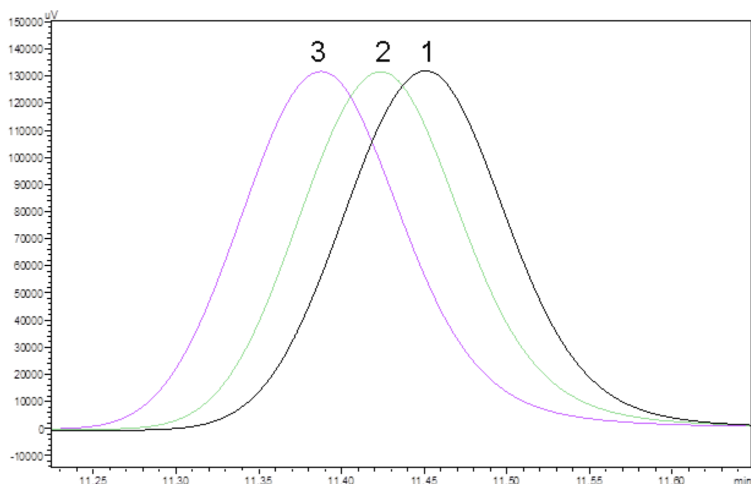
Pav. 4. Chromatogramos gautos analizuojant testinį mišinį 3, naudojant laboratorijoje pasigamintą (a) ir *Elite-1* (b) kolonėles. 1 – n-oktanas, 2 – 1-heksanolis. Chromatografinės sąlygos aprašytos ankstesniame skyriuje.

Kolonėlės ilgaamžiškumo įvertinimas

Kolonėlės terminio stabilumo testas buvo atliekamas kolonėlę kondicionuojant nuo 1 iki 40 valandų 200 °C temperatūroje. Kolonėlės ilgaamžiškumas buvo vertinamas pagal jos gebejimą išlaikyti analičių smailių aštrumą ir sulaikymo laikų stabilumą.

Pakartotinės testinių mišinių analizės po kondicionavimo parodė, kad testinių junginių sulaikymo laikų vertės sumažėjo. Tai labai akivaizdžiai buvo matoma testinio mišinio 2 chromatogramoje (5 pav.) vėliau eliuuojiemiems komponentams, tokiems kaip n-oktanas. n-Oktano sulaikymo laikas sumažėjo nuo 11,45 min (1 valanda kondicionavimo) iki 11,38 min (40 valandų

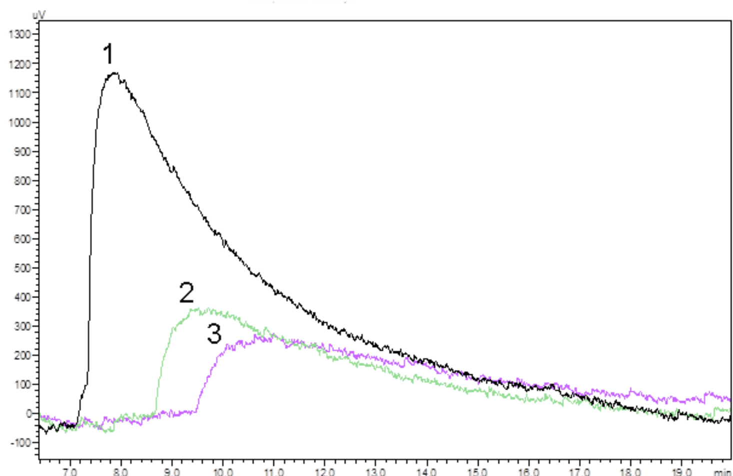
kondicionavimo). Toks sulaikymo laiko sutrumpėjimas indikuoja stacionarios fazės sluoksnio sumažėjimą.



Pav. 5. n-Oktano smailės chromatogramose, gautose analizuojant testinį mišinį 2 laboratorijoje pasigamintoje kolonėlėje po 1 (1), 20 (2) ir 40 (3) valandų kondicionavimo. Chromatografinės sąlygos aprašytos ankstesniame skyriuje.

Stipriu aktyvumu kolonėlės atžvilgiu pasižyminčios analitės po kondicionavimo dar labiau išplito ir sumažėjo smailės aukštis. Mažėjant stacionarios fazės sluoksniui, aktyvūs kolonėlės karkaso centrai tapo dar lengviau pasiekiami ir stipriau sąveikavo su analitėmis. Tai ypatingai akivaizdu 1-heksanolio smailėje. Po kondicionavimo smailė dar labiau išplatėjo ir pažemėjo (6 pav.).

Apskaičiuotas testinių mišinių komponentų smailių aukščių santykis inertiško n-oktano atžvilgiu po 1 ir po 40 kondicionavimo valandų. Duomenys yra pateikti 5 lentelėje. Neaktyvių komponentų benzeno, kumeno ir silpnai polinio chlorbenzeno smailių santykiai buvo beveik identiški nepriklausomai nuo kondicionavimo laiko. Iš kitos pusės stipriai aktyvaus anilino smailės su n-oktanu santykis stipriai sumažėjo po 40 kondicionavimo valandų. Toks sumažėjimas gali būti aiškinamas stacionarios fazės sluoksnio sumažėjimu kondicionuojant kolonėlę aukštoje temperatūroje.



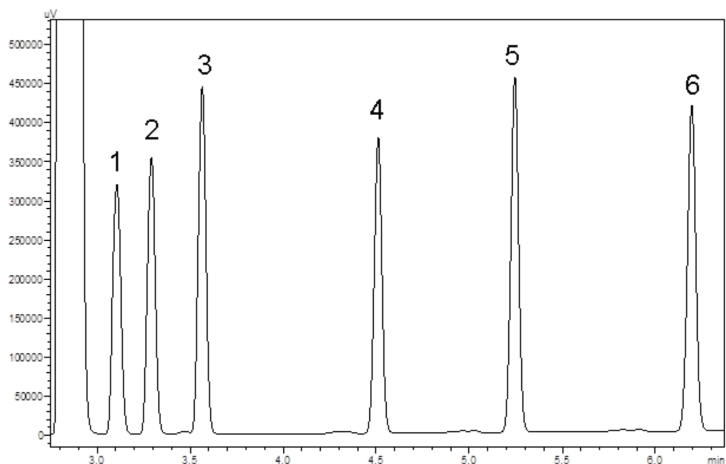
Pav. 6. 1-Heksanolio smailė mišinio 3 chromatogramoje, gautoje naudojant laboratorijoje pasigamintą kolonėlę po 1 (1), 20 (2) ir 40 (3) valandų kondicionavimo. Chromatografinės sąlygos aprašytos ankstesniame skyriuje.

5 lentelė. Testinių komponentų smailių aukščių santykis su n-oktanu. Analizės atliktos naudojant laboratorijoje pasigamintą kolonėlę po kondicionavimo esant 200 °C temperatūrai.

Junginys	1 valanda	40 valandų
Benzenas	1,027	1,033
Chlorbenzenas	1,022	1,040
Pentilacetatas	0,603	0,630
Kumenas	0,808	0,818
Anilinas	0,058	0,041
2-Butanonas	0,849	0,769
1-Heksanolis	0,005	0,001

Kolonėlės pritaikymo galimybės

Atsižvelgiant į aukščiau išdėstytus kolonėlės tyrimo duomenis galima teigti, kad laboratorijoje pagaminta kolonėlė turi trūkumų ir nėra tinkama analizuoti aktyvius junginius, tokius kaip aminai, alkoholiai, organinės rūgštys, aldehidai, ar ketonai. Kita vertus kolonėlė gali būti sėkmingai naudojama nepolinių, neaktyvių junginių chromatografinei analizei. 7 paveiksle pateiktoje chromatogramoje aiškiai matosi, kad alkanai gali būti sėkmingai atskirti esant simetriškoms alkanų smailėms ir trumpam analizės laikui.



Pav. 7. Tęstinio mišinio 5 chromatograma, gauta naudojant laboratorijoje pagamintą kolonėlę. 1 – n-oktanas, 2 – n-nonanas, 3 – n-dekanas, 4 – n-dodekanas, 5 – n-tridekanas, 6 – n-tetradekanas. Chromatografinės sąlygos aprašytos ankstesniame skyriuje.

Pabaigai galime teigti, kad kolonėlės pagamintos iš nerūdijančio plieno gali būti naudojamos aukštatemperatūrinėje chromatografijoje, tačiau metalo paviršius turėtų būti pasyvuotas, taip išvengiant aktyvių komponentų smailių degradavimo. Kita vertus, aktyvių junginių analizės parodė pasigamintos kolonėlės trūkumus. Gauti rezultatai parodė kolonėlės netinkamumą aktyvioms analitėms analizuoti.

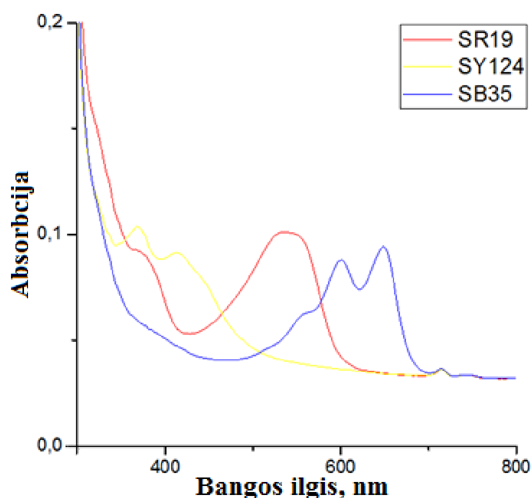
Tolesni darbai turėtų būti nukreipti į vidinių kolonėlės sienelių deaktyvavimą, taip gerinant kolonėlės inertiškumą. Taip pat norint padidinti kolonėlės šiluminį stabilumą ir ilgaamžiškumą, reikia padidinti stacionarios fazės paviršiaus susiejimą tarpusavyje (angl. crosslink).

3.2. Kuro dažiklių ir žymiklių nustatymas skysčių chromatografijos metodu

Detektoriaus bangos ilgio parinkimas

Kuro dažiklių ir žymiklių nustatymo skysčių chromatografijos metodu parengimas buvo atliekamas naudojant PerkinElmer Series 200 (PerkinElmes Instruments, USA) skysčių chromatografą, sukomplektuotą su UV/Vis detektoriumi. Šio tipo detektoriais galima registruoti vieno bangos ilgio šviesą. Prieš pasirenkant detekcijos bangos ilgį buvo padaryta spektrometrinė kuro dažiklių analizė nuo 400 iki 800 nm bangos ilgių diapazone.

Spektrometrinei analizei buvo paruošti individualūs dažiklių ir žymiklio tirpalai, kiekvieno koncentracija buvo 5 mg/L. Dažiklių ir žymeklio analizės spektrai pateikti 8 pav..



Pav. 8. Kuro dažiklių ir žymeklio spektrai, kiekvieno komponento koncentracija 5 mg/L

Iš 8 pav. galime matyti kad, optimalus bangos ilgis yra 535 nm dažikliui “Solvent Red – 19” ir 650 nm “Solvent Blue – 35” taip pat 390 nm žymikliui “Solvent Yellow – 124”.

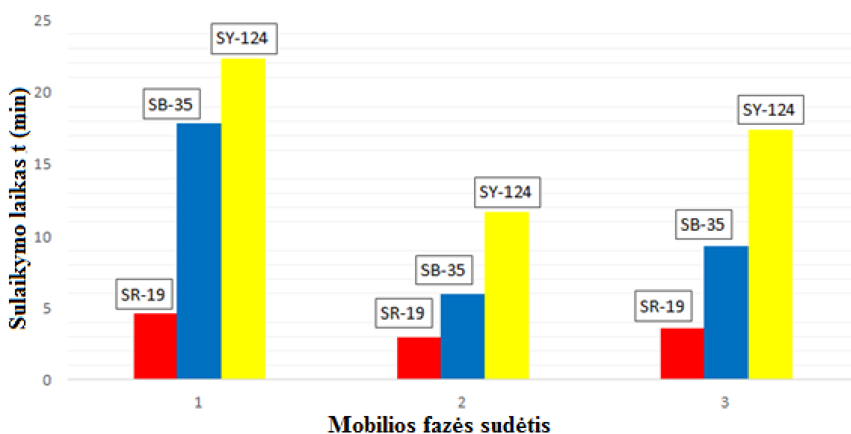
Chromatografinių sąlygų optimizavimas

Pradinėse dažiklių ir žymeklio metodo optimizavimo stadijose mobiliai fazei formuoti buvo pasirinkti heksanas, toluenas ir MTBE. Analizės pradžioje, iki 18 min mobilios fazės sudėtis buvo 75 % heksano ir 25 % tolueno, nuo 18 min į mobilią fazę buvo įvedamas MTBE. Tiesiškai didinama MTBE koncentracija nuo 0 % 18 min iki 8 % 23 min, tam kad eliuuoti labiausiai poliškus komponentus. Šioje analizėje analičių sulaikymo laikai buvo pakankamai ilgi, didinant MTBE kiekį mobilioje fazėje sulaikymo laikai trumpėjo, tačiau buvo reikalingas ilgesnis kolonėlės kondicionavimas.

Vėliau mobilios fazės komponentais buvo išmėginti toluenas ir etilacetatas. Mobiliai fazei formuoti buvo pasirinktas 98 % tolueno ir 2 % etilacetato, testuojant kaip bus sulaikomos analitės. Esant tokiai mobilios fazės sudėčiai nustatyta, kad dažiklis Solvent Red 19 buvo eliuuojamas kaip

nesulaikomas komponentas. Taipogi sulaikymo laikų skirtumas tarp "Solvent Blue – 35" ir "Solvent Yellow – 124" buvo pakankamai didelis.

Po šių bandymų buvo padaryta prielaida, kad heksanas prailgina "Solvent Red – 19" sulaikymą kolonėlėje, o etilacetatas trumpina "Solvent Blue – 35" ir "Solvent Yellow – 124 sulaikymą kolonėlėje. Remiantis šia prielaida buvo pasirinkta naudoti heksaną, tolueną ir etilacetatą kaip mobilios fazės komponentus. Panaudojus dažiklių ir žymeklio analizei mobilios fazės sudėtį 49,5/49,5/1 heksanas/toluenas/etilacetatas nustatyta, kad dažiklio „Solvent Red – 19" sulaikymo laikas pailgėjo, bet pailgėjo ir "Solvent Blue – 35" ir "Solvent Yellow – 124" sulaikymo laikai. Visi komponentų sulaikymo laikai pateikti 9 paveiksle. Tolimesniam metodo optimizavimui buvo pasirinkta paskutinioji mobilios fazės kompozicija.



Pav. 9. Analičių sulaikymo laikai esant skirtingoms mobilios fazės kompozicijoms. 1 – heksanas/toluenas/MTBE (69%/23%/8%); 2 – toluenas/etilacetatas (98%/2%); 3 – heksanas/toluenas/ etilacetatas (49,5%/49,5%/1%).

Metodo parametrų parinkimas

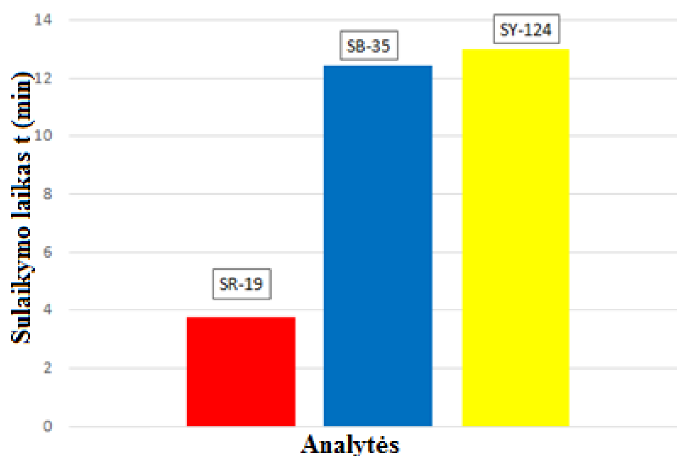
Įvairi kuro dažų ir žymeklio analizė parodė, kad geriausiai dažiklių ir žymeklio sulaikymo kolonėlėje kontrolei tinka heksanas, toluenas ir etilo acetatas. Naudojant gradientinę mobilios fazės programą pasiekiami geriausi rezultatai. Be to, buvo atlikta mobilios fazės optimizavimas siekiant išsiaiškinti optimalų analičių eliuavimo laiką, bei įvertintas kolonėlės kondicionavimo laikas.

Analizei pritaikyta ir optimizuota mobilios fazės sudėtis pateikta 6 lentelėje .

6 lentelė. Mobilios fazės sudėtis ir programa.

Žingsnis	Laikas (min)	Mob.f. v (mL/min)	Heksanas (%)	Toluenas (%)	Etilacetatas (%)
Kondicionavimas	7,0	1,0	30	70	0
1	2,0	1,0	30	70	0
2	8,0	1,0	0	93	7
3	5,0	1,0	0	93	7

Nustatytas minimalus kolonėlės kondicionavimo prieš analizę laikas buvo 7 min. Bendras analizės laikas buvo 22 min. Analičių sulaikymo laikai pateikti 10 paveiksle.



Pav. 10. Kuro dažiklių ir žymiklio sulaikymo laikai

Identifikavimo riba (LOD) ir nustatymo riba (LOQ)

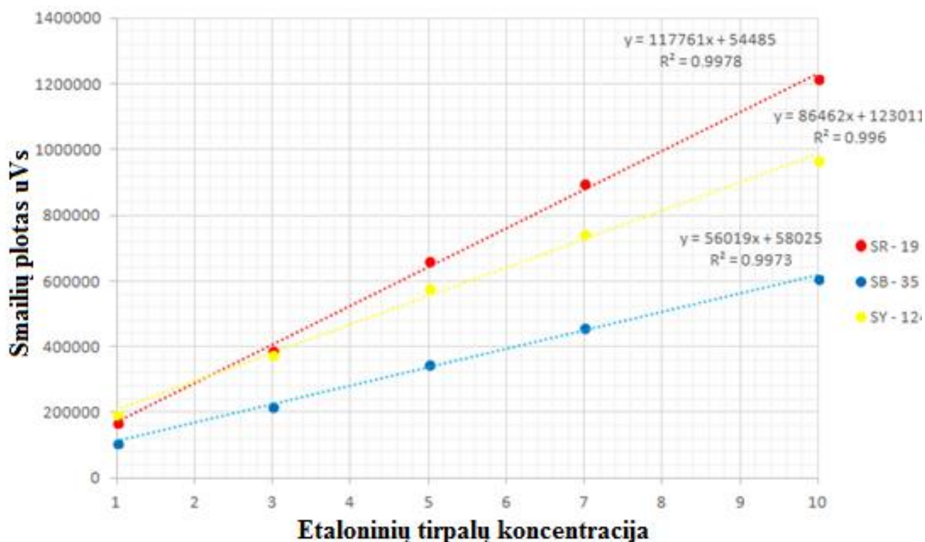
Atlikus mobilios fazės optimizavimą, buvo išmatuotas kolonėlės efektyvumas, tai yra teorinių lėkštelių skaičius N ir lėkštelės aukštis H:

$$N = 5{,}54 \times \left(\frac{3{,}75}{(3{,}827 - 3{,}702)}\right)^2 = 4986 \qquad (3)$$

$$H = \frac{4984}{250} = 19{,}94\ (mm) \qquad (4)$$

Siekiant išsiaiškinti kuro dažiklių ir žymeklio aptikimo ir nustatymo ribas buvo paruošti standartiniai tirpalai. Paruoštame pirminiame tirpale buvo po 5 g/L kiekvieno komponento. Iš pirminio tirpalo buvo paruošti etaloniniai

tirpalai, kuriuose komponentų koncentracijos buvo nuo 1 iki 10 mg/L. Naudojant šiuos tirpalus buvo parengtos kalibracinės kreivės, o iš jų ekstrapoliacijos būdu buvo išskaičiuotos aptikimo ribos (LOD) ir nustatymo ribos (LOQ) vertės. Analičių aptikimo vertės koncentracija buvo mažesnė nei 1 mg/kg, o analičių nustatymo vertė 1,5 mg/kg.



Pav. 11. Kuro dažiklių ir žymeklio kalibracijos kreivės.

Taip pat atliktas metodo atsparumo, išgavos, glaudumo ir tikslumo įvertinimas. Tiesiškumo matavimas buvo atliktas 2,5-50 mg/L diapozone, ilgalaikis matavimų rezultatų glaudumas buvo mažiau nei 15%. Kalibracinės kreivės ir išmatuoti metodo parametrai yra pateikiami 11 pav. ir 7 lentelėje.

7 lentelė. Metodo validavimo parametrai

Analitė	t_R, min	Tiesės lygtis	r^2	LOD, mg/L	LOQ, mg/L	RSD %
SR – 19	3,75	y = 117761x - 54485	0,998	0.,56	1,88	2,1
SB – 35	12,04	y = 86462x - 123011	0.997	0.63	2.10	5.0
SY - 124	12,44	y = 56019x - 58025	0.996	0.77	2.55	1.0

Kuro mėginių analizės

Parengtas metodas ištestuotas analizuojant realius mėginius. Šie mėginiai buvo gauti iš vietinės kompanijos, kuri atlieka kuro dažymą ir žymėjimą. Buvo išanalizuotos dvi kuro rūšys, kurioms taikomi lengvatiniai mokesčiai. Pirmoji kuro rūšis buvo nudažyta dažikliu “Solvent Red – 19” ir žymekliu “Solvent Yellow – 124”. Antroji kuro rūšis buvo nudažyta “Solvent Blue – 35” ir “Solvent Yellow – 124”. Analizių rezultatai pateikti 8 lentelėje.

8 lentelė. Realių dažytų kuro mėginių analizės rezultatai

Mėginys	Dažas	Išmatuota c (mg/L)	Privaloma c* (mg/L)	Žymeklis	Išmatuota c (mg/L)	Privaloma c* (mg/L)
Žalios spalvos kuras	SB - 35	0,61	5.0	SY – 124	4,42	8.5 – 12.7
Raudonos spalvos kuras	SR - 19	0,45	6.0	SY - 124	4,63	8.5 – 12.7

*- ***privaloma koncentracija*** *– žemiausia dažo koncentracija pagal LR Energetikos ministerijos įsakymą.*

IŠVADOS

1. Šiame darbe buvo pagaminta nerūdijančio plieno kolonėlė. Kolonėlės, padengtos PDMS skysta faze, charekteristikų ištyrimui buvo pasiūlytas testavimo mišinys. Taip pat buvo nustatyta, kad komercinė Elite-1 kolonėlė buvo efektyvesnė, jos minimalus teorinės lėkštelės aukštis buvo 0,295 mm, tuo tarpu laboratorijoje pagamintos kolonėlės lėkštelės aukštis buvo 0,386 mm.
2. Kolonėlė gerai eliuavo nepolinius junginius: benzeną, n-oktaną, kumeną ir chlorbenzeną. Šių smailių asimetriškumas buvo artimas 1.
3. Kolonėlės inertiškumas nepakankamas eliuti įvairaus poliškumo junginiams, turintiems stiriai išreikštą dipolio momentą, bazines ar rūgštines savybes.
4. Kolonėlė pasižymėjo vidutiniu terminiu stabilumu. Atlikus 40 val kondicionavimą esant 200 °C n-heksano sulaikumo laikas pakito mažiau nei 0,5 %.
5. Tolimesni kolonėlės efektyvinimo darbai turi būti nukreipti į kolonėlės deaktyvavimo gerinimą. Kolonėlės ilgaamžiškumo ir terminio stabilumo pagerinimui reiktų atlikti stacionarios fazės surišimą.
6. Kolonėlės savybių pakanka kad galima būtų panaudoti ją kolonėlės-termostato tyrimams.
7. Buvo atliktas skysčių chromatografijos analitinio metodo lengvatinio apmokestinimo kuro dažikliams ir žymekliui optimizavimas. Optimizavimo metu nustatyta, kad kuro dažiklių detekcijai UV/VIS detektoriumi nustatyti efektyviausia naudoti 535 nm bangos ilgį, kai detektuojamas dažiklis “Solvent Red – 19”; 650 nm bangos ilgį, kai detektuojamas dažiklis “ Solvent Blue – 35”; 390 nm bangos ilgį, kai detektuojamas žymeklis “ Solvent Yellow – 124”.
8. Atliktas metodo validavimas kurio metu nusatytos kuro dažiklių Solvent Red – 19, Solvent Blue – 35 ir žymiklio Solvent Yellow – 124 aptikimo ribos, nustatymo ribos, atsikartojamumas.
9. Optimizuotas metodas panaudotas realių kuro pavyzdžių analizei. Šių analizių metu nustatyta 0,45 mg/L SR -19 koncentracija ir 4,63 mg/L SY-124 koncentracija raudoname lengvatinio apmokestinimo kuro mėginyje, taip pat 0,61 mg/L SB-35 koncentracija ir 4,42 mg/L SY-124 koncentracija žaliame lengvatinio apmokestinimo kure mėginyje.

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I would like to sincerely thank Professor A. Kareiva who tirelessly encouraged me to achieve this goal and his ability to connect things what are not look like possible to connect

I would also like to thank the supervisor S. Šakirzanov for his patience and professionalism, Associate Professor E. Naujalis for his support, Professor V. Vičkačkaitė for her help, as well as colleagues A. Markevičius and A. Sadaunykas for being.

LIST OF PUBLICATION

6.1. Articles in Journals

1. A.Zolumskis; V. Vičkačkaitė; A. Kosinskaitė; A. Markevičius; A. Sadaunykas; E.Naujalis; S.Šakirzanovas. An assessment of the in-house made stainless steel capillary GC column. Chemija. 2017. vol. 28. No. 2. P. 117–124
2. A. Markevičius, A. Zolumskis, A.Sadaunykas, B.Knašienė, A. V. Claramunt, S. Šakirzanovas, E.Naujalis. Determination of dyes and marker in diesel using high performance liquid chromatography. Chemija . 2018. Vol. 29. No. 2. p. 121–126

6.2. Attended Conferences

1. A. Zolumskis, A. Sadaunykas, A. Markevičius Stainless steel capillary columns for gas chromatography. Preparation and usage. Open Readings 2017 Vilnius
2. A. V. Claramunt, A. Paškevičius, J. Švedienė, E. Gudeliūnaitė, S. Kiverytė, R.Petrauskaitė, L. Griškevičius, A. Zolumskis, E. Naujalis Thermal desorption methodology for volatile organic compounds (VOC's) identification: Possible application in invasive fungal infections. Open Readings 2017 Vilnius
3. A.Sadaunykas, A. Zolumskis, E. Naujalis Analyte focusing and enrichment on gas chromatography column Open Readings 2020 Vilnius

1

An assessment of the in-house made stainless steel capillary GC column

A.Zolumskis; V. Vičkačkaitė; A. Kosinskaitė; A. Markevičius; A. Sadaunykas; E.Naujalis; S.Šakirzanovas

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An assessment of the in-house made stainless steel capillary GC column

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To assess the performance of an in-house made stainless steel capillary column coated with a methyl polysiloxane stationary phase, demanding test mixtures containing benzene, n-octane, chlorobenzene, pentyl acetate, isopropylbenzene (cumene), benzenamine (aniline), 2-butanone, 1-hexanol and propionic acid have been suggested. The gas chromatographic analysis of the mixtures reveals that the column lacks inertness towards active analytes and can be used only for the analysis of the mixtures containing nonpolar compounds.

Keywords: stainless steel column, gas chromatography, test probes

INTRODUCTION

To assess the performance of capillary columns for gas chromatography, quality control test probes are used. These probes ensure that the columns have been properly deactivated, contain the correct amount of the stationary phase, and have the same relative retention as the last column purchased [1–3]. In 1978, Grob and Grob for column assessment proposed a test mixture composed of various classes of organic components including hydrocarbons, fatty acid methyl esters, acids, bases and alcohols [4]. The Grob test mixture with subsequent refinements became the benchmark for column testing [5–8]. In the tests, inert probes serve to calculate chromatographic efficiency and as indicators of the efficacy of the injection process. Any tailing or lost response of the acidic probe indicates that the column is basic in nature. Poor peak behaviour of the base indicates that the column is acidic. The alcohol will give an indication if there is any oxygen damage or if there are any exposed silanols. If the peak shapes for all of these compounds are symmetrical, then the column is considered to be inert towards them [1].

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The choice of the test probes can either highlight or mask the deficiencies of the column. Ultimately, in order to reveal the drawbacks of the columns, in test mixtures more demanding compounds are often used. As a rule, the compounds have less sterically hindered active groups, have lower boiling points and thus are eluted at lower temperatures [1].

At present, the most widely used capillary gas chromatographic columns are wall coated fused-silica open tubular columns. Those columns are prepared from specially purified silica with a minimal amount of metal oxides, thus demonstrate low reactivity towards sample compounds. Recently, a new generation of stainless steel capillary columns began to gain an increasing interest as a special treatment of the internal surface of the tubing makes the surface as inert as deactivated fused silica. Stainless steel capillary columns can be used at higher temperatures (450 °C) than fused silica columns (standard rating is 360 °C) and do not break under stress [9, 10]. However, commercial columns are rather expensive. Thus it should be of great interest to prepare less expensive columns with improved properties compared with the available commercial columns.

In this work, the first trial to create and to develop an in-house made stainless steel capillary column with a methyl polysiloxane stationary phase is described. Demanding test probes were selected and the column properties were evaluated and compared with the commercially available column Elite-1 (PerkinElmer).

EXPERIMENTAL

Reagents and solutions

Pentane (99%), hexane (99%), dichloromethane (99.8%), n-octane (99%), n-nonane (99%), n-decane (99%), n-dodecane (99%), n-tridecane (99%), n-tetradecane (99%), benzene (99%), chlorobenzene (99.9%), pentyl acetate (99%), cumene (isopropylbenzene) (98%), aniline (benzenamine) (99.5%), 1-hexanol (98%), 2-butanone (99%), and propionic acid (99%) and dimethylpolysiloxane (PDMS) (99%) were purchased from Sigma-Aldrich (Germany).

Stock solutions of individual compounds (n-octane, n-nonane, n-decane, n-dodecane, n-tridecane, n-tetradecane, benzene, chlorobenzene, pentyl acetate, cumene, aniline, 1-hexanol, 2-butanone and propionic acid (10 mg ml^{-1} each)) were prepared in dichloromethane. Six working solutions were prepared in dichloromethane:

1. Benzene, n-octane, chlorobenzene, pentyl acetate, cumene and aniline (1 mg ml^{-1} each).
2. 2-Butanone and n-octane (1 mg ml^{-1} each).
3. 1-Hexanol and n-octane (1 mg ml^{-1} each).
4. Propionic acid and n-octane (1 mg ml^{-1} each).
5. n-Octane, n-nonane, n-decane, n-dodecane, n-tridecane and n-tetradecane (1 mg ml^{-1} each).
6. n-Decane (1 mg ml^{-1}).

Instrumentation

The chromatographic analysis was performed on a Shimadzu GC-2010 Plus gas chromatograph equipped with an AOC-20i auto injector and a flame ionization detector. The following gas chromatographic columns were used: PerkinElmer fused silica Elite-1 (crossbond 100% dimethyl polysiloxane) capillary column (30 m × 0.32 mm ID, film thickness 1 μm) and stainless steel 100% dimethyl polysiloxane capillary column (30 m × 0.32 mm ID) prepared in our laboratory.

Stainless steel capillary column preparation

The 304 grade stainless steel capillary of 0.32 mm ID and 0.5 mm OD has been acquired at local suppliers. For column preparation the 30-meter capillary was used. PDMS was dissolved in hexane with the ratio 1:10 by volume. The prepared solution was pumped through a stainless steel capillary. Excess of the solution was removed by inert gas pressure. The column was dried overnight under inert gas flow.

Gas chromatographic conditions

Helium was employed as a carrier gas with a column flow rate of 1.2 ml min^{-1}. The injector temperature was held at 250 °C. Injection was performed in a split mode with a split ratio of 10:1. The flame ionization detector temperature was held at 250 °C. Helium gas was used as make up gas at 30 ml min^{-1} flow rate. The hydrogen flow rate was 40 ml min^{-1}, the air flow rate was 400 ml min^{-1}.

The oven temperatures were as follows: 1) for working solutions 1, 2 and 6 the oven temperature was 100 °C; 2) for working solutions 3 and 4 the oven temperature was 60 °C, and 3) the temperature programmed mode was used for solution 5: 170 °C for 1 min, from 170 to 200 °C at 7 °C min^{-1} and held at 200 °C for 3 min.

RESULTS AND DISCUSSION

Optimisation of mobile phase flow rate

Column efficiency depends on the mobile phase flow rate. At the optimal flow rate the theoretical plate height is smallest. Thus, first of all, the optimal mobile phase flow rate through a laboratory prepared column and a commercial PerkinElmer Elite-1 column was determined. The working standard solution of n-decane in dichloromethane was used. The helium flow rate varied from 0.5 to 7 ml min^{-1}, and the theoretical plate height H was calculated employing the equations [11]

$$N = 5.54\left(\frac{t_R}{w_{0.5}}\right)^2 \quad (1)$$

and

$$H = \frac{L}{N}, \quad (2)$$

where N is the number of theoretical plates, t_R is the retention time of n-decane, $w_{0.5}$ is the peak width (at 0.5 of height) of n-decane, L is the column length.

As can be seen from the results presented in Fig. 1, for the in-house developed stainless steel column as well as for Elite-1 the optimum mobile phase flow rate is 1.2 ml min^{-1}.

The results also demonstrate that the commercial Elite-1 is more efficient with a minimal plate height of 0.295 mm, meanwhile for our prepared column the minimal H is 0.386 mm. The both columns were coated with the same stationary phase PDMS, and in the both cases the same carrier gas helium was used. The internal diameter of Elite-1 is 0.25 mm, however, our column has 0.32 mm ID. This could be the reason of better efficiency of Elite-1. One more probable reason of the difference in the efficiencies of columns is a less uniform stationary phase film on the stainless steel column.

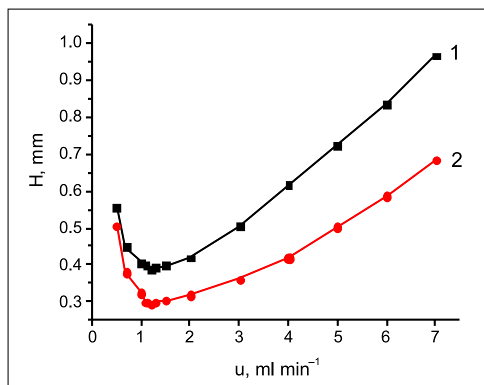

Fig. 1. Effect of the mobile phase flow rate on the plate height for in-house made (1) and Elite-1 (2) columns

Test probe selection

The activity of a column is determined by the measurement of any deleterious effects the column has towards challenging compounds. These interactions may be acidic, basic, or strongly hydrogen bonding. Poor behaviour is exhibited by tailing peaks or reduced peak response. Both of these behaviours lead to an inaccurate calculation of the peak areas and, consequently, an inaccurate quantification of the active compounds of interest [1].

Probes selected for column testing should demonstrate if the column is thermally stable, properly deactivated, if it contains the correct amount of a stationary phase.

As it was mentioned in the Introduction Section, the choice of individual compounds in the test probe can either highlight or mask the deficiencies of a column with respect to activity. By selecting undemanding probes, column activity can go undetected. Ideally test compounds should be molecules with low molecular weights, low boiling points, and no steric shielding of the active groups. These characteristics allow the probative portion of the test molecule to penetrate and fully interact with the column's stationary phase and surface [1].

For GC column evaluation the Grob test mixture is available commercially. However, the Grob test mixture is a quite undemanding probe. For example, it contains 2,6-dimethylphenol as an organic acid to test column basicity and 2,6-dimethylaniline as a base to test column acidity. Those compounds are weak probes as the active sites of the molecules are shielded by two methyl groups on the phenyl ring.

In order to prepare a more demanding probe we examined more than 30 test probe candidates and selected nine compounds, namely, benzene, n-octane, chlorobenzene, pentyl acetate, cumene, aniline, 2-butanone, 1-hexanol and propionic acid. In order to elute the compounds from the column in a reasonable time but, on the other hand, to use as low column temperature as possible and to avoid peak overlapping, four different mixtures containing those compounds were prepared (Table 1). 2,6-Dimethylaniline from the Grob test mixture was substituted by aniline, and 2,6-dimethylphenol was substituted by propionic acid. The molecules of aniline and propionic acid have no steric shielding of the active groups thus can fully interact with the column's stationary phase and surface. Also, in order to make the test more demanding, we substituted 1-octanol by 1-hexanol. As the chain length in the alcohol decreases, the molecules become less hydrocarbon-like and thus more active. At elevated temperatures the interactive forces of the analytes are diminished, thus weakening their usefulness as test probes [1]. Taking this under consideration and seeking to prepare a demanding test, methyl esters of decanoic, undecanoic and dodecanoic acids from the Grob test mixture were substituted by pentyl acetate.

Table 1. **Test mixtures in dichloromethane**

Mixture	Compounds (10 mg ml^{-1} each)
1	Benzene, n-octane, chlorobenzene, pentyl acetate, cumene, aniline
2	2-Butanone, n-octane
3	1-Hexanol, n-octane
4	Propionic acid, n-octane

Column inertness evaluation

The inertness of the column was evaluated based on the peak shapes of test mixture analytes. When the column was evaluated using mixture 1, good peak shapes were observed only for nonpolar analytes (Fig. 2a). The peaks of benzene, n-octane and cumene were symmetric and comparable with those obtained on the commercial column Elite-1 (see Table 2 and Fig. 2). With the increase of compounds polarity, the peak asymmetry increased to 1.17 for the slightly polar chlorobenzene and to 2.6 for the more polar pentyl acetate. The tailed peaks of polar molecules indicate a possible dipole–dipole interaction of the analytes with the column walls. Like esters, ketones are polar molecules and thus also interact with the column walls as it is evident from the peak of 2-butanone (Fig. 3a and Table 2). A very poor performance was observed for aniline. The aniline

Table 2. **Peak tailing measured at 10% of peak height**

Compound	In-house column	Elite-1
Benzene	1.10	1.05
n-Octane	1.06	1.02
Chlorobenzene	1.17	1.01
Pentyl acetate	2.60	0.99
Cumene	1.08	0.97
Aniline	11.0	0.94
2-Butanone	3.01	1.11
1-Hexanol	11.1	0.99

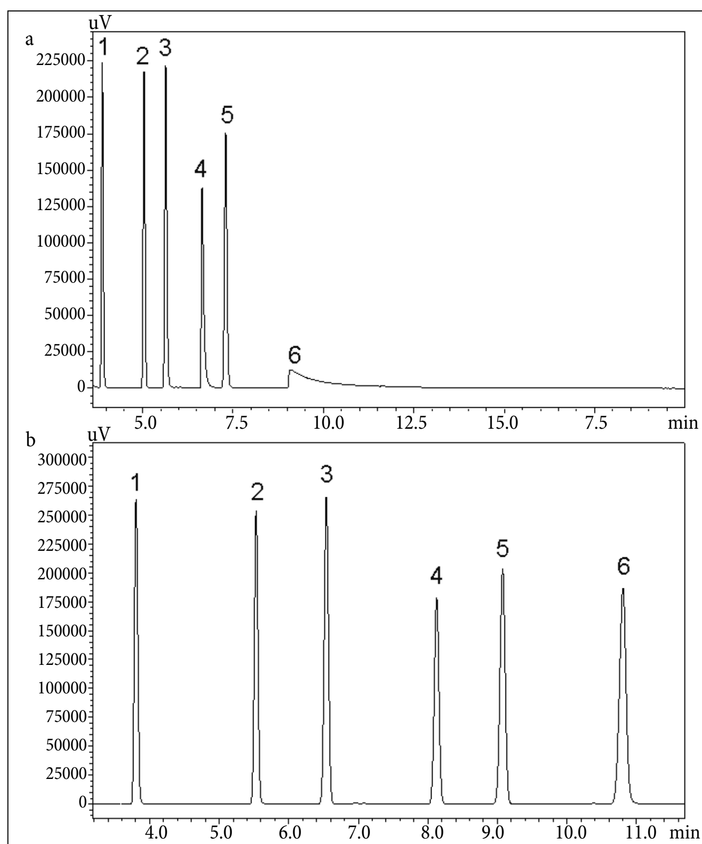

Fig. 2. Chromatograms of mixture 1 obtained using an in-house made stainless steel column (a) and Elite-1 (b). 1 is benzene, 2 is n-octane, 3 is chlorobenzene, 4 is pentyl acetate, 5 is cumene, 6 is aniline. Chromatographic conditions are described in the Experimental Section

peak tailing at 10% height was 11. Aniline is a basic analyte thus its tailing peak indicates the presence of acidic active sites in the column. Aniline is able to form hydrogen bonds, thus its deteriorated peak is an evidence of a probable presence of hydrogen-bonding sites in the column. A very asymmetric peak of 1-hexanol could also be explained by hydrogen bond formation with the active sites in the column (Fig. 4a and Table 2). Propionic acid was particularly strongly retained in the column

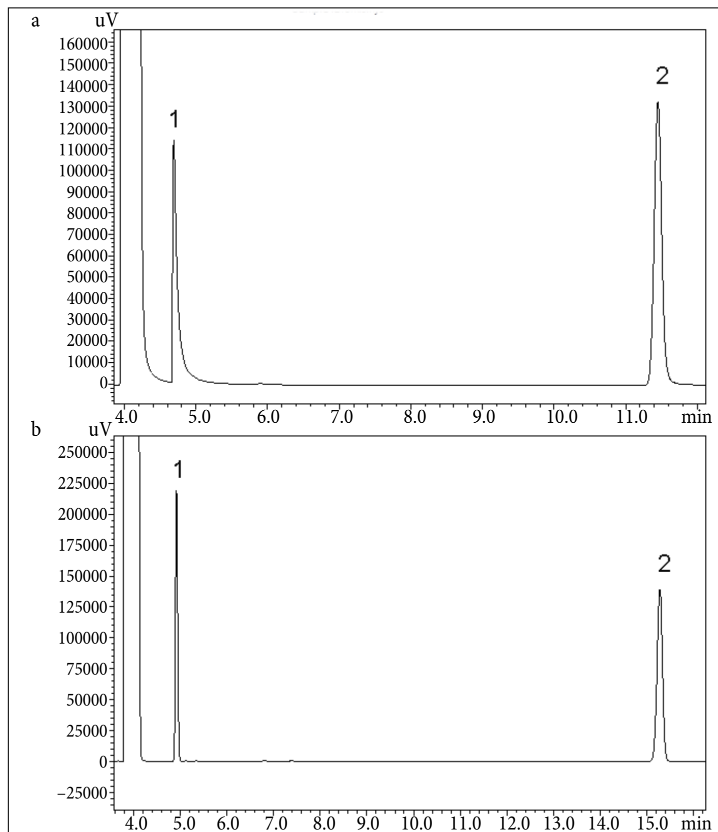

Fig. 3. Chromatograms of mixture 2 obtained using in-house made stainless steel (a) and Elite-1 (b) columns. 1 is 2-butanone, 2 is n-octane. Chromatographic conditions are described in the Experimental Section

and its peak was not observed in the chromatogram. In comparison, the peaks of the analytes obtained on the commercial column Elite-1 at the same chromatographic conditions were symmetric (see Figs. 2b, 3b, 4b and Table 2). The only exception was propionic acid that gave a severely fronting peak.

Column longevity evaluation

The column's thermal longevity test was accomplished after conditioning the column from 1 to 40 hours at 200 °C. The column longevity was characterised by the loss of responses of the active analytes and the shift of retention times.

Re-testing of the column after conditioning revealed that the retention times shortened. It is particularly evident for later eluting peaks, e.g. for n-octane in the chromatogram of test mixture 3 (Fig. 5). The n-octane retention time decreased from 11.45 min (1 hour conditioning) to 11.38 min (40 hours conditioning) indicating a significant stationary phase loss.

Strongly active analytes normally give lower peak heights and responses because of their adsorption onto the column active sites. With the stationary phase loss the column active sites became more easily assessable and interact stronger with the active analytes. It is particularly evident for the 1-hexanol peak that after conditioning became even broader and significantly lower (Fig. 6).

Peak height ratios between the tested compounds and an inert n-octane were calculated after conditioning of the column for 1 hour and 40 hours. As can be seen from the results presented in Table 3, the peak ratios for inactive benzene, cumene and slightly polar chlorobenzene were almost identical independently on the conditioning time. On the other hand, the peak ratios of strongly active aniline and 1-hehanol significantly decreased after 40 hours of conditioning. This can be attributed to the stationary phase loss and consequent

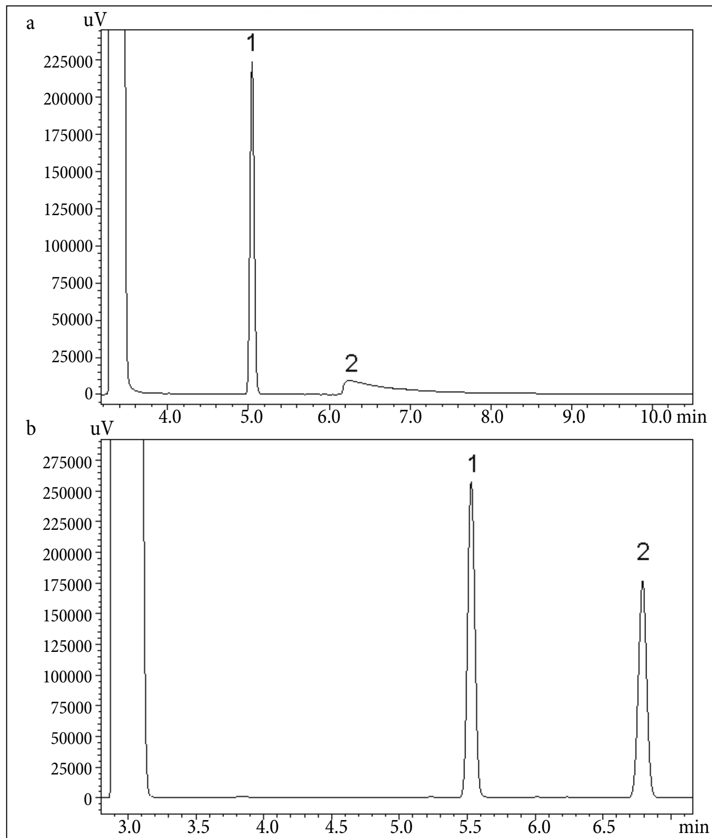

Fig. 4. Chromatograms of mixture 3 obtained using in-house made stainless steel (a) and Elite-1 (b) columns. 1 is n-octane, 2 is 1-hexanol. Chromatographic conditions are described in the Experimental Section

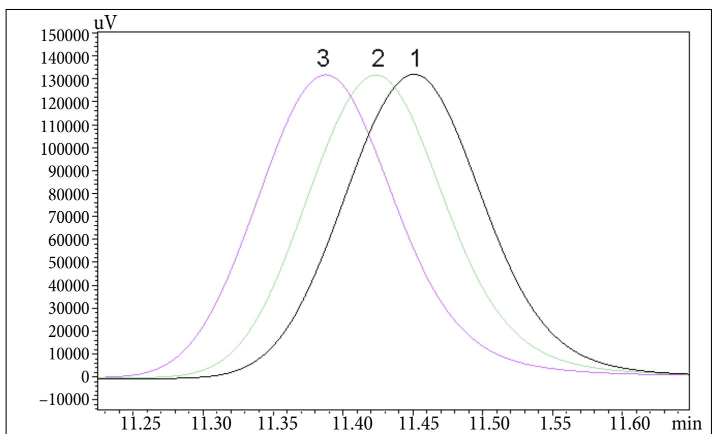

Fig. 5. n-Octane peak in the chromatograms of mixture 2 obtained using an in-house made stainless steel column after 1 (1), 20 (2) and 40 (3) hours of conditioning. Chromatographic conditions are described in the Experimental Section

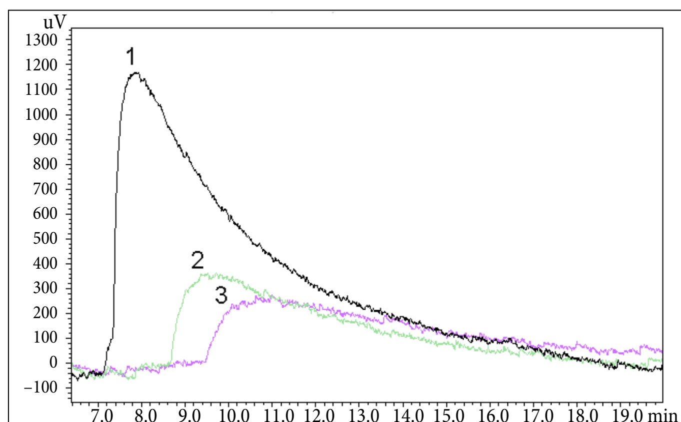

Fig. 6. 1-Hexanol peak in the chromatograms of mixture 3 obtained using an in-house made stainless steel column after 1 (1), 20 (2) and 40 (3) hours of conditioning. Chromatographic conditions are described in the Experimental Section

Table 3. **Peak height ratios between test compounds and n-octane after conditioning of the in-house column at 200 °C**

Compound	1 hour	40 hours
Benzene	1.027	1.033
Chlorobenzene	1.022	1.040
Pentyl acetate	0.603	0.630
Cumene	0.808	0.818
Aniline	0.058	0.041
2-Butanone	0.849	0.769
1-Hexanol	0.005	0.001

deterioration of the column inertness with conditioning at high temperature.

Column application

Concerning the above presented column evaluation it is evident that the column has significant drawbacks and would not fit properly for the analysis of the samples containing active analytes such as amines, alcohols, organic acids, aldehydes or ketones. On the other hand, the column could be applied for the determination of nonpolar and inactive compounds. The chromatogram presented in Fig. 7 demonstrates that alkanes could be successfully separated with good peak shapes and a short analysis time.

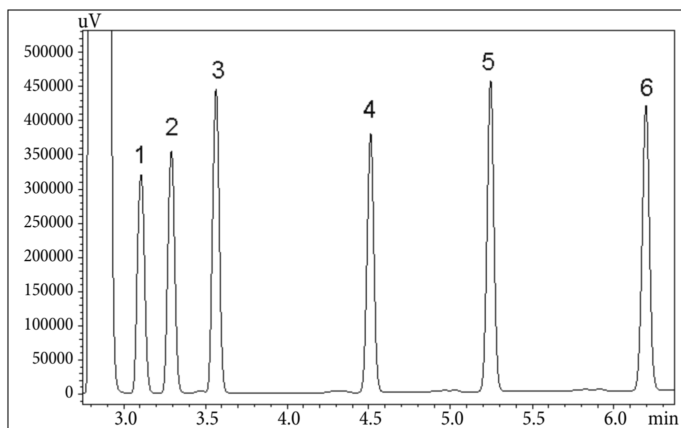

Fig. 7. Chromatogram of mixture 5 obtained using an in-house made stainless steel column. 1 is n-octane, 2 is n-nonane, 3 is n-decane, 4 is n-dodecane, 5 is n-tridecane, 6 is n-tetradecane. Chromatographic conditions are described in the Experimental Section

CONCLUSIONS

Stainless steel columns are probably the best choice for high-temperature chromatography. However, the metal surface with active sites is a matter of great concern when dealing with active analytes. On the other hand, active analytes enable in a great extent to reveal the deficiencies of a column. In the presented work, demanding test probes have been suggested for the evaluation of an in-house prepared stainless steel capillary column coated with a PDMS stationary phase. The results demonstrated the poor column performance towards active analytes indicating the presence of active sites on the column surface. The column can be applied only for the analysis of mixtures containing nonpolar compounds. Further work should be directed towards the deactivation of inner column walls and thus to the improvement of column inertness. In order to enhance thermal stability and longevity of the column, bonded or crosslinked stationary phases should be used.

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NAUJOS LABORATORIJOJE PAGAMINTOS NERŪDIJANČIO PLIENO KAPILIARINĖS DUJŲ CHROMATOGRAFINĖS KOLONĖLĖS SAVYBIŲ ĮVERTINIMAS

Santrauka

Laboratorijoje pagamintai nerūdijančio plieno kapiliarinei dujų chromatografinei kolonėlei, padengtai metilpolisiloksano nejudriąja faze, įvertinti pasiūlyti testavimo mišiniai, sudaryti iš benzeno, n-oktano, chlorbenzeno, pentilacetato, kumeno, anilino, 2-butanono, 1-heksanolio ir propioninės rūgšties. Šių mišinių dujų chromatografinė analizė parodė, kad kolonėlė nepakankamai inertiška aktyvioms analitėms, tačiau gali būti sėkmingai taikoma analizuoti mėginiuose esančius nepolinius junginius.

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Determination of dyes and marker in diesel using high performance liquid chromatography

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Determination of dyes and marker in diesel using high performance liquid chromatography

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A fast, precise and accurate high performance liquid chromatography method has been developed for the determination of dyes (Solvent Red 19 and Solvent Blue 35) and a marker (Solvent Yellow 124) in diesel. Separation was carried out on a 250 × 4.60 mm Agilent Zorbax Rx-SIL column (5 µm particle size). Detection was done in a visible wavelength range. The best performance of fuel dye separation and the shortest retention times were achieved when using hexane, toluene and ethyl acetate as a mobile phase. During this research the eluent composition and the elution gradient were optimized consequently that helped to perform the analysis within 15 min. The developed method was applied for the analysis of real samples of dyed diesel fuel. Preparation of the samples for the analysis simply consisted of filtering through a 0.45 µm filter previous to direct injection of the sample into the HPLC system for analysis.

Keywords: HPLC, fuel, dyes, diesel, marker, Solvent Red 19, Solvent Blue 35, Solvent Yellow 124

INTRODUCTION

Fuel dyes provide colour to fuel, and could be identified visually. Fuel dyes can also be used as some fuel markers, they must be of different colour and exact concentration that can be determined by analytical methods. Fuel dyes are used to differentiate fuel with differently applied taxes. It is important that dyed fuel stands out by its colour from the fuel which is not dyed and does not have lower taxes [1].

Dyes are added to fuel which is used for heating in agriculture, aquaculture and commercial fishing in inland waters. Also, without dyes there are fuel markers which are used to mark the fuel. Fuel markers are the same materials as fuel dyes can change fuel colour, but must be in an exact concentration and could be determined with analytical methods. Fuel markers are used to track the origin of the fuel.

According to the Order on Fuels of the Ministry of Energy of Lithuania, in Lithuania there are two types of fuel dyes: Solvent Red 19 (SR 19) and Solvent Blue 35 (SB 35), and one marker: Solvent Yellow 124 (SY 124) [1]. Furthermore, there are fixed concentrations of the dyes and the marker used in fuels with lower taxes. Fuel dying and marking must be carried out using a specific methodology [1, 6]. However, an exact chemical analysis method which allows the determination of

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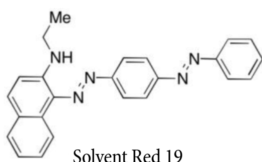
various fuel dyes, markers and, also, their concentration effectively in one run is not yet available [2–10]. The difference and variety of analytical methods suitable for the determination of fuel dyes and markers have appeared for all EU countries using different combinations of dyes and markers. There are suggestions for electrochemical [4, 5], spectrophotometric [6, 7], gas chromatographic [8, 9] and HPLC [10] analytical methods to determine chosen fuel dyes or markers. But the variety of fuel dyes and markers set up the problem if country has not adopted a method for their own dyes and markers because there is no suitable analytical method to determine all fuel dyes or markers in one run.

The aim of this study was to develop, optimize and adopt a HPLC method which would allow sensitive detection and accurate determination of the concentrations of the dyes and the marker in fuels (SR 19 and SB 35; and the marker: SY 124) using single injection.

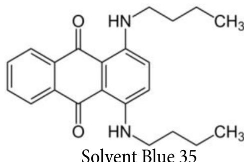
Structural formulas of fuel dyes and marker

1. Solvent Red 19 (N-ethyl-1-(phenyldiazophenylazo)-2-aminonaphtalen):



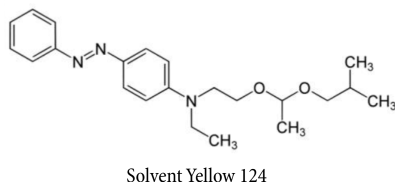
Solvent Red 19

2. Solvent Blue 35 (1, 4-bis (butylamino)-9,10 anthraquinone):



Solvent Blue 35

3. Solvent Yellow 124 (N-ethyl-N-[2-(1-izobutoxyethoxy) ethyl]-4-phenildiazenyl) aniline



Solvent Yellow 124

EXPERIMENTAL

Reagents and solutions

Toluene (HPLC grade), hexane (HPLC grade), MTBE (methyl tret-buthyl ether) (99.8%), Solvent Red 19 (N-ethyl-1-(phenyldiazophenylazo)-2-aminonaphtalen) (96%), Solvent Blue 35 (1,4-bis(butylamino)-9,10-anthraquinone) (98%), Solvent Yellow 124 (N-ethyl-N-[2-(1-izobutoxyethoxy) ethyl]-4-phenildiazenyl) aniline) (98%) were obtained from Sigma-Aldrich (Germany). Ethyl acetate (HPLC grade) was purchased from Merck (Germany). Stock solutions of the fuel dyes and the marker (SR 19, SB 35 and SY 124) (10 mg/L each) were prepared in toluene. Working solutions of the fuel dyes and the marker were prepared by dilution of the stock solution with toluene. All solutions were stored in the dark at 4°C.

Dyed fuel samples were purchased from the local petrol stations.

Sample preparation

The real samples of dyed fuel were filtered through 0.45 μm PTFE Chromafil Xtra PTFE-45/25 filters (Macherey-Negel, Germany) filters. After filtering, the samples were transferred into 2 mL chromatographic vials.

Instrumental

The chromatographic analysis was performed on a Liquid Chromatograph PerkinElmer Series 200 (PerkinElmer Instruments, USA) using UV/Vis spectrophotometric detection. A 200 μL volume injection loop was used for automated injection. The HPLC system was equipped with a Zorbax Rx-SIL column (250 mm × 4.60 mm ID, sorbent particle size 5 μm) (Agilent Technologies, USA). A 150 μL high pressure mixer (PerkinElmer Instruments, USA) was used.

Parameters of HPLC analysis

Real samples were filtered through a 0.45 μm PTFE filter right into a 2 mL vial. The vials with the prepared samples were placed into an autosampler for injection. The best separation of analytes with the shortest retention times was achieved while using a mobile phase consisting of hexane, toluene and ethyl acetate. A constant mobile phase flow of 1 mL/min was set. A gradient composition of the mobile phase was used to get the best results: firstly, 7 min the column was conditioned with a hexane/toluene (30/70 vol.%) mixture. The first 2 minutes after the injection the mobile phase composition was the same as used for preconditioning; after 8 min the mobile phase composition changed to the toluene/ethyl acetate (93/7 vol.%) solution; in the last step the isocratic mobile phase composition was held for 5 min. The total time of the analysis of dyes and markers in fuel was 22 min. The HPLC column must be preconditioned for 7 min before every analysis to remove ethyl acetate from the column.

RESULTS AND DISCUSSION

Selection of detection wavelengths

The sample analysis was performed with a PerkinElmer Series 200 (PerkinElmer Instruments, USA) equipped with a UV/Vis detector. The absorption wavelength of each

analyte should be optimized in order to improve the analysis sensitivity. The spectrophotometric analysis was performed for each dye and marker solution using a solution with a 5 mg/L concentration. The results presented in Fig. 1 show that the highest absorption is at 535 nm for SR 19, 650 nm for SB 35, and 390 nm for the marker SY 124. By setting the UV/Vis detector to those wavelengths for each analyte, the highest detection sensitivity was achieved. Our detector could record only two signals at two different wavelengths, consequently it was decided to use the reference wavelength mode to monitor the third one.

All measurements were made with a two-channel UV/Vis detector. In the first channel, the 535 nm main wavelength was chosen to detect SR 19, and the 390 nm reference wavelength was chosen to detect SY 124 so this peak in all chromatograms is shown as a negative peak. In the second channel, the 650 nm main wavelength was chosen to detect SB 35.

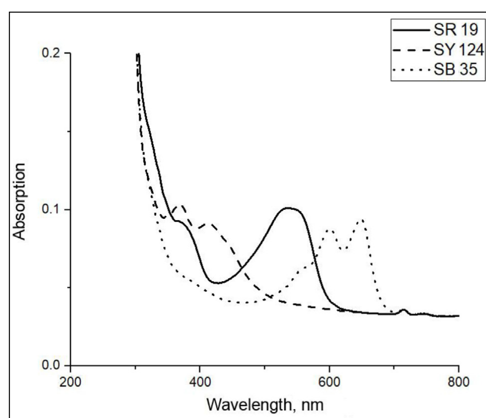


Fig. 1. Results of the spectrophotometric analysis of the fuel dyes and the marker (concentration of each analyte 5 mg/L)

Optimization of separation conditions

As the first eluent composition, hexane, toluene and MTBE were chosen. However, this composition is appropriate only for SR 19 and SY 124, with no results for SB 35. The analysis started with the hexane and toluene (75/25 vol.%) eluent composition, after 18 min MTBE was added to the mobile phase for the next 5 min, so at the end of the analysis the eluent composition was hexane, toluene and MTBE (71/21/8 vol.%). Using this methodology, the retention time of analytes was very long. Furthermore, increasing the MTBE concentration in the mobile phase decreases the measurement repeatability and prolongs the preconditioning time of the HPLC column.

A different eluent composition could be used for the detection of SB 35 and SY 124. Using the mobile phase of toluene and ethyl acetate (98/2 vol.%) in an isocratic mode was not suitable for SR 19. After few injections it was clear that SR 19 was eluted with the dead volume. Increasing or decreasing the ethyl acetate concentration has no effect on SR 19 retention. Also there was a large retention time difference between SB 35 and SY 124.

After series of separations using different eluents, it seems that ethyl acetate is a good eluent additive for the dyes SR 19 and SB 35 and the marker SY 124. In order to achieve the shortest analyte retention times the eluent consisting of hexane/toluene/ethyl acetate (49.5/49.5/1 vol.%) was tried. This analysis showed that by using less ethyl acetate in the eluent dyes SR 19 and SB 35 and the marker SY 124 retentions can be managed. All retention times with different mobile phases are shown in Fig. 2. For further analysis, the last mobile phase composition was chosen.

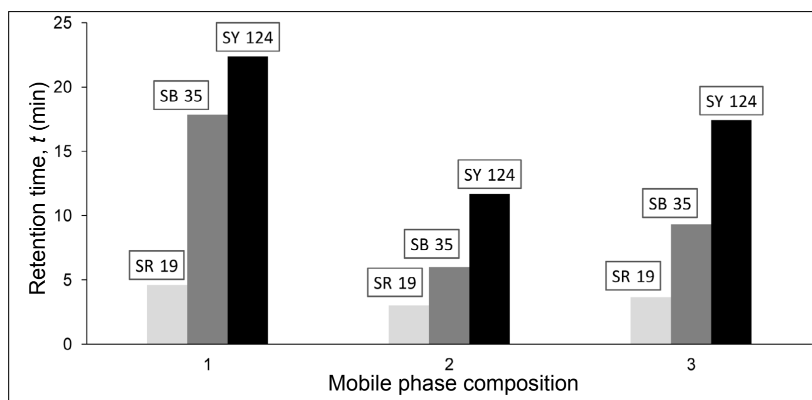


Fig. 2. Comparison of the retention times of analytes according to the composition of different mobile phases: 1, hexane/toluene/MTBE (71%/21%/8%); 2, toluene/ethyl acetate (98%/2%); 3, hexane/toluene/ethyl acetate (49.5%/49.5%/1%)

The optimized and adopted mobile phase composition for the analysis of the dyes and the marker in fuel is shown in Table 1. The experiment showed that the shortest retention and the best separation of analytes was when the three different solvents were used: hexane, toluene and ethyl acetate. Moreover, using a gradient elution with these solvents the best results can be obtained. In addition to that, analyses were performed to find out the shortest time for column conditioning (i.e. to eliminate all ethyl acetate from it). The optimized conditioning time was found to be 7 min. The total time of the analysis is 22 min.

Table 1. **Mobile phase composition for the analysis**

Step	Time, min	Flow rate, mL/min	Hexane, %	Toluene, %	Ethyl acetate, %
Conditioning	7.0	1.0	30	70	0
1	2.0	1.0	30	70	0
2	8.0	1.0	0	93	7
3	5.0	1.0	0	93	7

Determination of method parameters

The main chromatographic parameters were determined and calculated. Column efficiency could be evaluated according to the theoretical plate number (N) and its height (H):

$$N = 5.54 \times \left(\frac{t_R}{w_{0.5}} \right)^2 \cdot \text{eq.}, \quad (1)$$

$$H = \frac{L}{N} \cdot \text{eq.} \quad (2)$$

All the calculations were made measuring the SR 19 standard solution with the 5 mg/L concentration. The theoretical plate number (N) and its height (H) were 4986 and 0.05014 mm, respectively.

After the mobile phase composition and separation optimization, five standard solutions with a concentration range from 1 to 10 mg/L were prepared. All results of the calibration curves are shown in Fig. 3 and Table 2.

Limit of detection (LOD) and limit of quantification (LOQ)

The linearity of the response of the chromatographic system was tested by recording chromatograms for different concentrations in the range 1–10 mg/L, using the previously optimal conditions and with the standard solution corresponding to each point of the calibration curve. Each calibration curve was constructed using five concentration levels, prepared by the serial dilution of working standard solutions. From Fig. 3 it can be seen that a good linearity was achieved, with a correlation coefficient (*r*) greater than

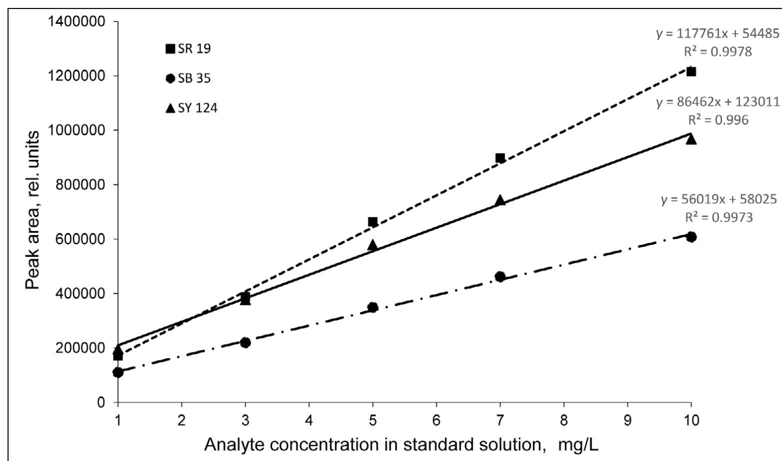


Fig. 3. Calibration curves of the analytes measured using series of the standard solutions prepared in hexane

Table 2. **Main analytical characteristics**

Analyte	Retention time, min	Line equation	r^2	LOD, mg/L	LOQ, mg/L	Repeatability RSD, % ($n = 7$)
SR 19	3.8	$y = 117761x + 54485$	0.998	0.56	1.88	2.1
SB 35	12.0	$y = 86462x + 123011$	0.997	0.63	2.10	5.0
SY 124	12.4	$y = 56019x + 58025$	0.996	0.77	2.55	1.0

0.99 for all the compounds within the calibration range used. The parameters of the regression equations are given in Table 2. The standard deviation of the y-intercept (S_A) of the regression line calculated for each compound was used to calculate the limit of detection (LOD) and quantification (LOQ), as 3 and $10*S_A/m$, respectively, where m is the slope of the calibration curve. The limits of detection using this relationship were between 0.56 and 0.77 mg/L, the limits of quantification were between 1.88 and 2.55 mg/L shown in Table 2, showing that the method is capable of determining dye concentrations at the levels that must be found in real samples.

Analysis of diesel samples

The optimized method was successfully applied for the measurements of real samples – diesel fuel. Two different samples of coloured diesel were measured using the developed method. The concentrations of the dyes and the marker for fuel colouring were determined. The samples were received from the company engaged in fuel dying and marking. Two types of fuel with low taxes were analysed. The first was the red colour fuel suitable for heating systems which dyed with Solvent Red 19 and marked using Solvent Yellow 124. The second was the green colour fuel suitable for agriculture or aquaculture aggregates which dyed with Solvent Blue 35 and marked using Solvent Yellow 124. The results of the analysis are shown in Table 3 and Figs. 4–6. The first analysis of red dyed fuel showed that the in-house developed method was fully suitable for the determination of Solvent Red 19 and Solvent Yellow 124, but the dye and the marker used for dying fuel are not pure from their isomers or mixed up with another dyes and markers of the same colour. Consequently, a similar situation has been obtained with the data of the analysis of the second sample which should be dyed with Solvent Blue 35 and marked with Solvent Yellow 124.

Table 3. **Results of the analysis of real samples**

Sample	Dye	Measured concentration, mg/L	Officially compulsory concentration*, mg/L	Marker	Measured concentration, mg/L	Officially compulsory concentration*, mg/L
Green colour diesel fuel	SB 35	0.61	5.0	SY 124	4.42	8.5–12.7
Red colour diesel fuel	SR 19	0.45	6.0	SY 124	4.63	8.5–12.7

* Oficially compulsory concentration, the lowest concentration of the necessary dye or marker in fuel, as required according to the regulation of the Ministry of Energy [1].

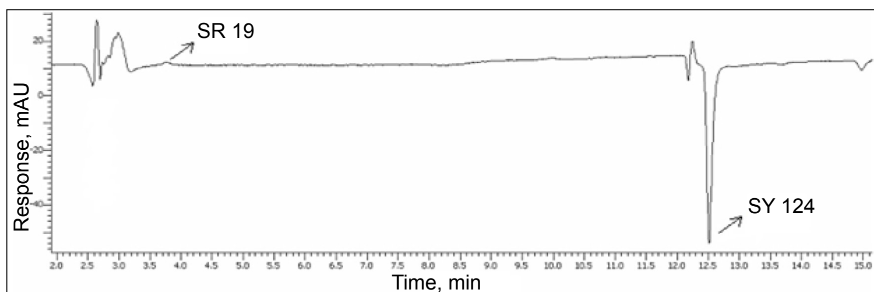


Fig. 4. Chromatogram of the red coloured fuel (dyed with SR 19 and marked with SY 124). Eluent composition: hexane, toluene, ethyl acetate; detection at 535 nm as the main wavelength and at 390 nm as the reference wavelength; injection volume 20.0 μL

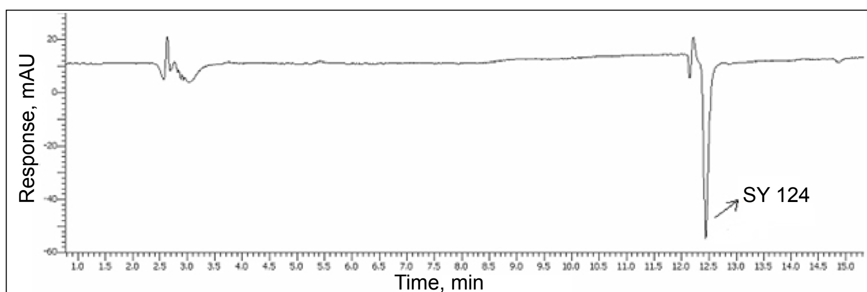


Fig. 5. *C*hromatogram of the green coloured fuel (dyed with SB 35 and marked with SY 124). Eluent composition: hexane, toluene, ethyl acetate; detection at 535 nm as the main wavelength and at 390 nm as the reference wavelength. Injection volume 20.0 μL

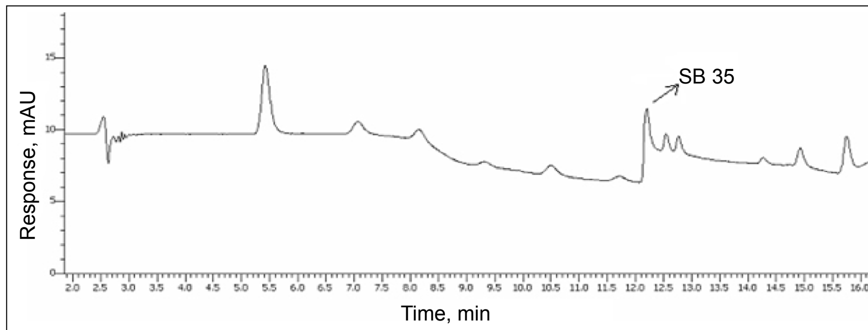


Fig. 6. Chromatogram of the green coloured fuel (dyed with SB 35 and marked with SY 124). Eluent composition: hexane, toluene, ethyl acetate; detection at 640 nm as the main wavelength. Injection volume 20.0 µL

CONCLUSIONS

The developed method was applied for the analysis of dyed diesel fuel samples. The method is able to separate and to detect the analytes which are used according to the national regulation for colouring and marking of the low taxed diesel fuels in one run. Using this method concentrations of 0.45 and 4.63 mg/L of SR 19 and SY 124, respectively, were determined in the red dyed diesel, and 0.61 and 4.42 mg/L of SB 35 and SY 124, respectively, in the green dyed diesel sample. It is possible to adopt this HPLC method for other types of dyes and markers in real samples.

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DAŽIKLIŲ IR ŽYMIKLIO NUSTATYMAS DYZELINIAME KURE EFEKTYVIOSIOS SKYSČIŲ CHROMATOGRAFIJOS METODU

Santrauka
Paruoštas, ištirtas ir optimizuotas dyzeliniame kure naudojamų dažiklių („Solvent Red – 19“, „Solvent Blue – 35“) ir žymiklio („Solvent Yellow – 124“) nustatymo metodas. Optimalios šio metodo sąlygos, kai iš pradžių judrioje fazėje taikant gradientinį režimą heksanas ir toluenas sudaro 70 % / 30 %, nuo antros analizės minutės iki analizės pabaigos judrios fazės sudėtį sudaro toluenas ir etilacetatas 93 % / 7 %, o judrios fazės srauto greitis 1 mL/min. Iš viso analizė trunka 22 min. Analitės detektuojamos, kai: SR-19 – 535 nm; SB-35 – 640 nm; SY-124 – 390 nm. Metodas pritaikytas dviejų tipų (raudonai nudažyto ir žaliai nudažyto) realių kuro mėginių analizei. Sėkmingai nustatytos koncentracijos dviejuose dyzelinio kuro mėginiuose siekė 0,61 mg/L SB-35; 0,45 mg/L SR–19 ir atitinkamai 4,42 bei 4,63 mg/L žymiklio SY–124. Šios nustatytos koncentracijos neatitiko minimalių reikalaujamų koncentracijų pagal LR Energetikos ministerijos ministro įsakymą [1].

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