CHEMIJA. 2017. Vol. 28. No. 2. P. 117–124

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

Audrius Zolumskis[1, 2],

Vida Vičkačkaitė[1*],

Augustė Kosinskaitė[1],

Audrius Markevičius[1, 3],

Audrius Sadaunykas[1, 2],

Evaldas Naujalis[1, 3],

Simas Šakirzanovas[1]

[1] *Faculty of Chemistry and Geosciences, Vilnius University, Naugarduko St. 24, LT-03225 Vilnius, Lithuania*

[2] *Department of Nuclear Research, Center for Physical Sciences and Technology, Savanorių Ave. 231, LT-02300 Vilnius, Lithuania*

[3] *Department of Metrology, Center for Physical Sciences and Technology, Savanorių Ave. 231, LT-02300 Vilnius, Lithuania*

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].

* Corresponding author. E-mail: vida.vickackaite@chf.vu.lt

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.

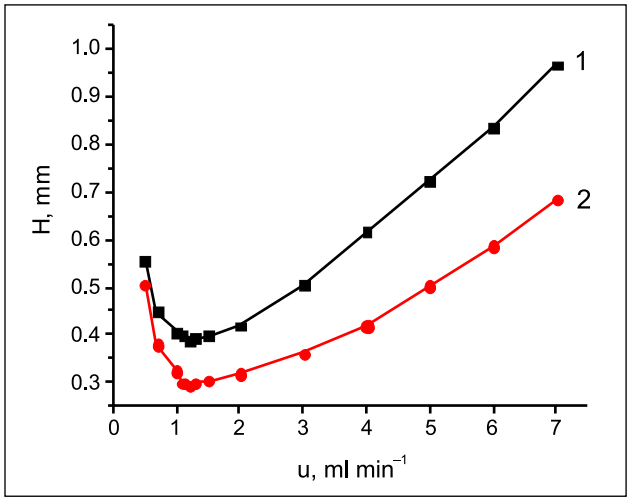

Fig. 1. Effect of the mobile phase flow rate on the plate height for in-house made (1) and Elite-1 (2) columns

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

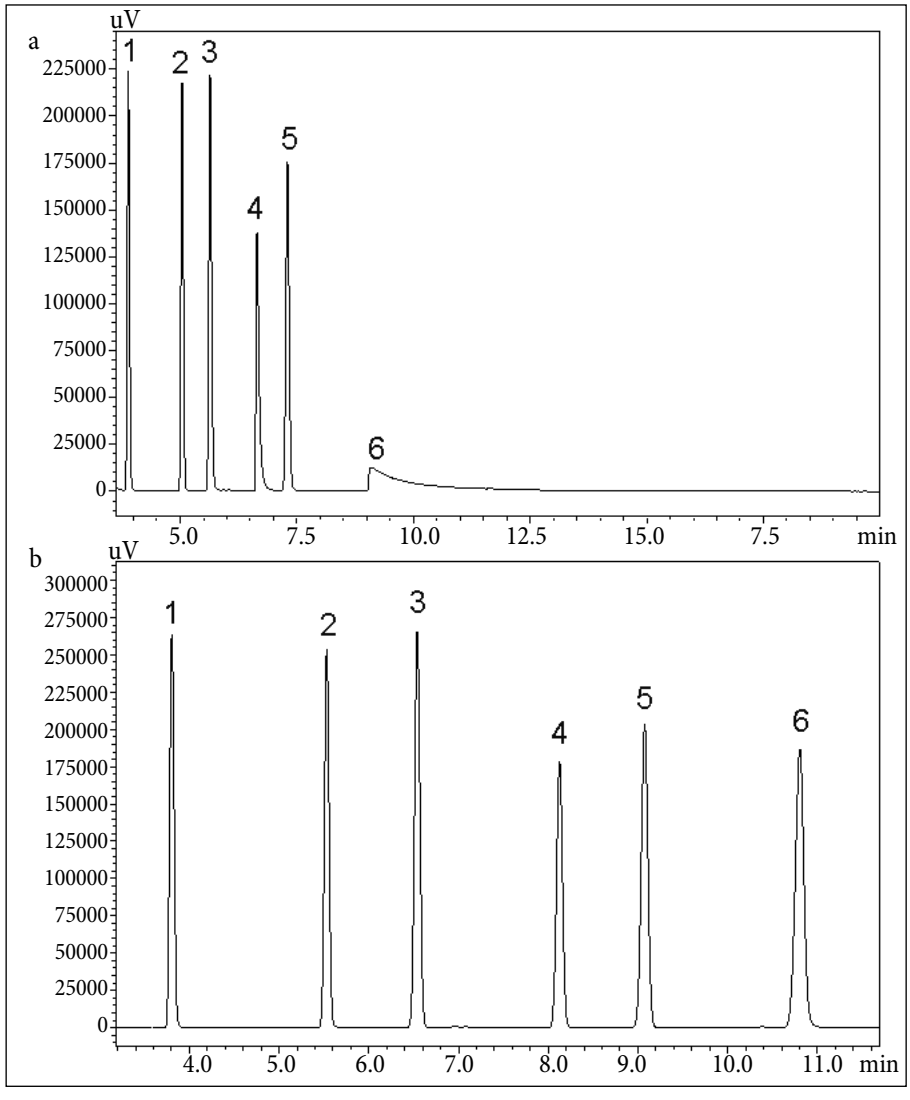

Fig. 2. Chromatograms of mixture 1 obtained using an in-house made stainless steel column (a) and Elite-1 (b). 1 is benzene, 2 is n-octane, 3 is chlorobenzene, 4 is pentyl acetate, 5 is cumene, 6 is aniline. Chromatographic conditions are described in the Experimental Section

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

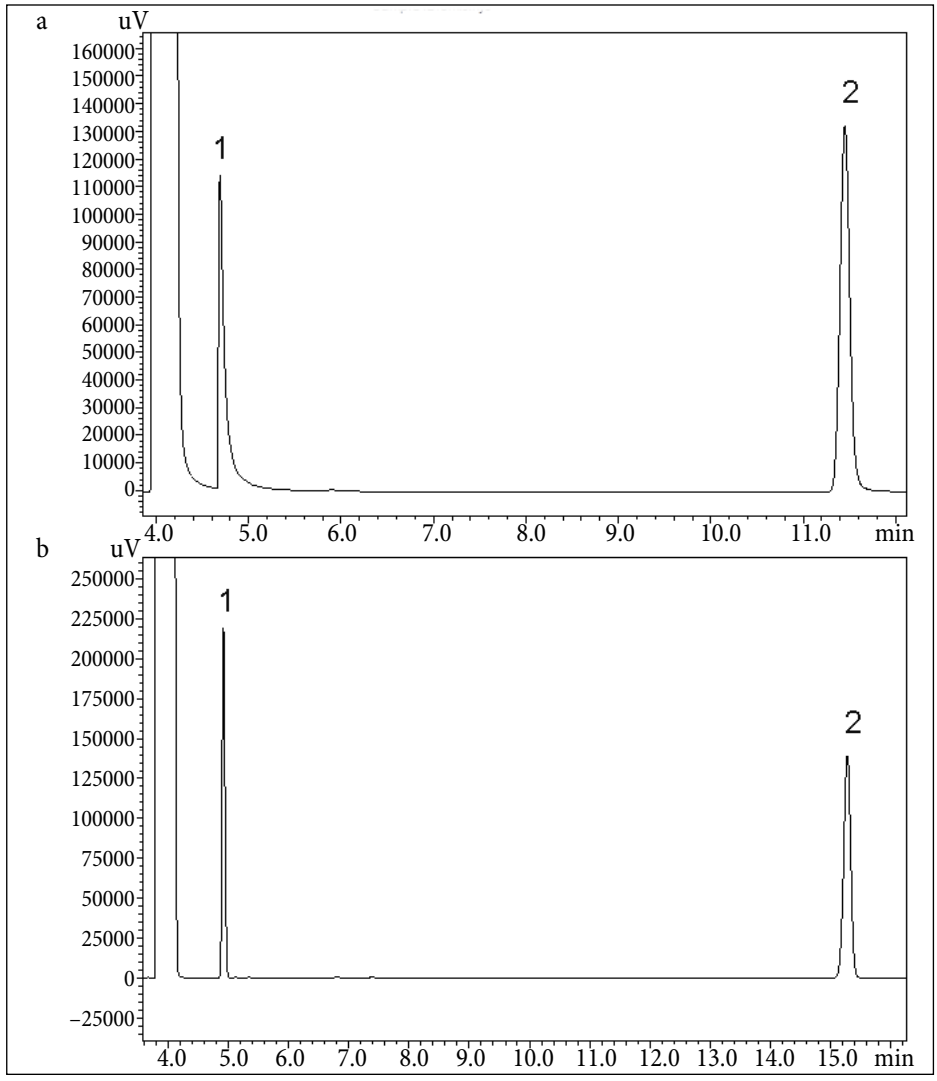

Fig. 3. Chromatograms of mixture 2 obtained using in-house made stainless steel (a) and Elite-1 (b) columns. 1 is 2-butanone, 2 is n-octane. Chromatographic conditions are described in the Experimental Section

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

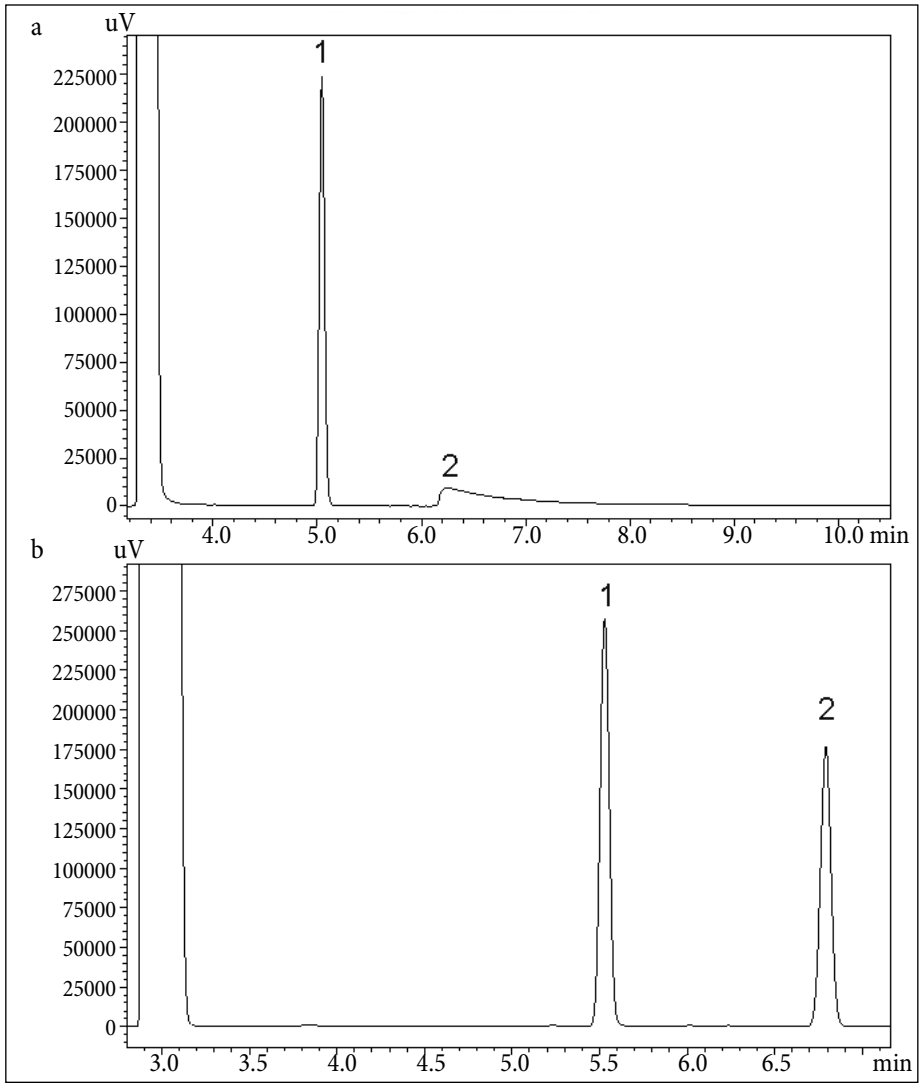

Fig. 4. Chromatograms of mixture 3 obtained using in-house made stainless steel (a) and Elite-1 (b) columns. 1 is n-octane, 2 is 1-hexanol. Chromatographic conditions are described in the Experimental Section

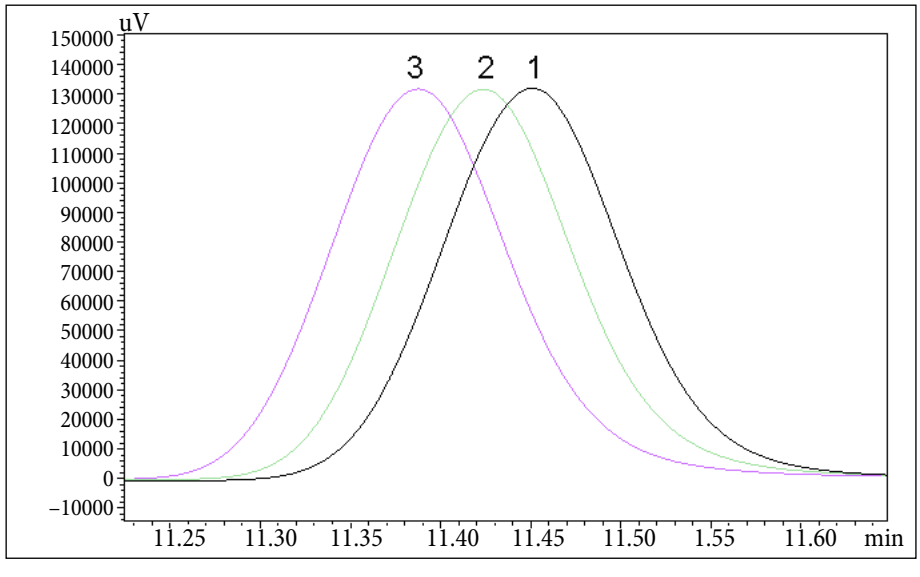

Fig. 5. n-Octane peak in the chromatograms of mixture 2 obtained using an in-house made stainless steel column after 1 (1), 20 (2) and 40 (3) hours of conditioning. Chromatographic conditions are described in the Experimental Section

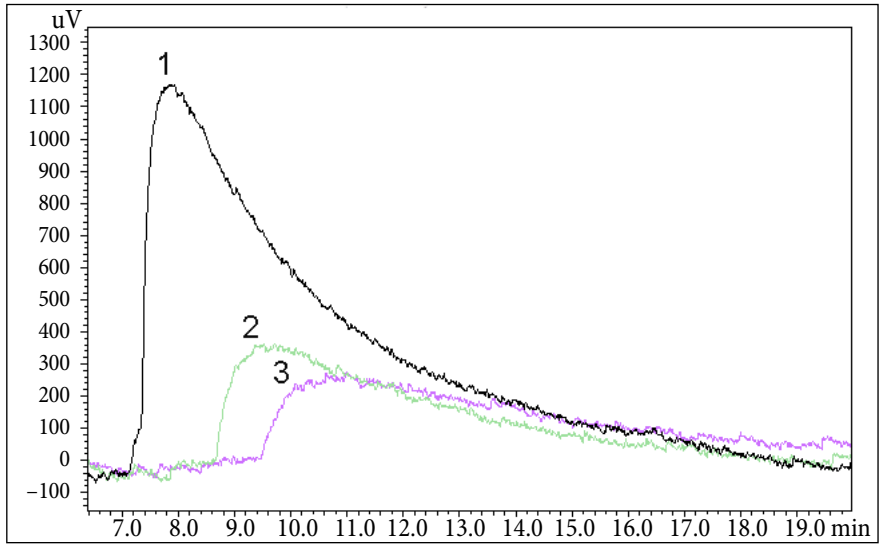

Fig. 6. 1-Hexanol peak in the chromatograms of mixture 3 obtained using an in-house made stainless steel column after 1 (1), 20 (2) and 40 (3) hours of conditioning. Chromatographic conditions are described in the Experimental Section

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.

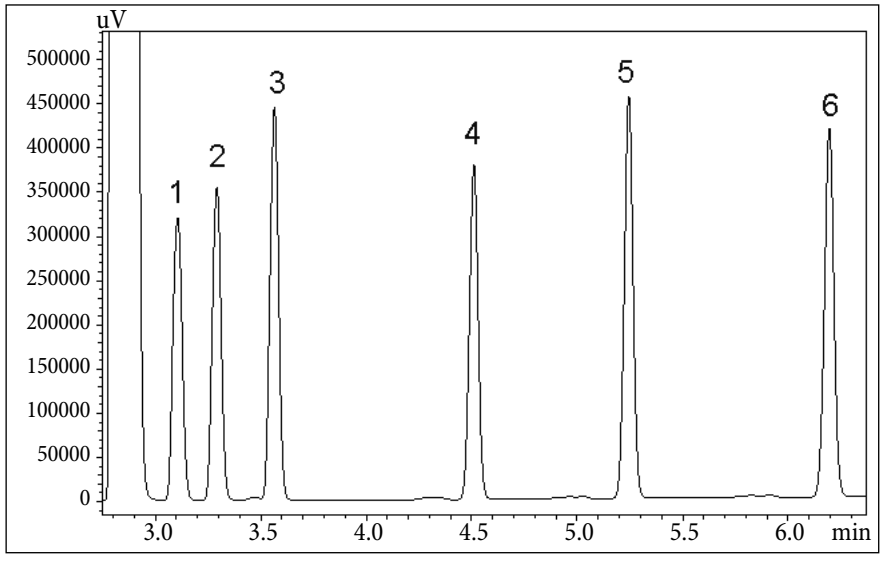

Fig. 7. Chromatogram of mixture 5 obtained using an in-house made stainless steel column. 1 is n-octane, 2 is n-nonane, 3 is n-decane, 4 is n-dodecane, 5 is n-tridecane, 6 is n-tetradecane. Chromatographic conditions are described in the Experimental Section

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.

Received 29 March 2017
Accepted 14 April 2017

References

1. *Agilent J & W Ultra Inert GC Columns: A New Tool to Battle Challenging Active Analytes*, Technical Overview, Publication number 5989-8665EN, Agilent Technologies, Inc. (2008).
2. E. Kuhn, M. Hastings, A. Broske, *5989-1687EN*, Agilent Technologies, Inc. (2004).
3. N.-A. Dang, A. K. Vickers, *A New PEG GC Column with Improved Inertness Reliability and Column Lietime*, Competitive Comparison, Publication number 5991-6683EN, Agilent Technologies, Inc. (2016).
4. K. Grob, G. Grob, K. Grob, *J. Chromatogr. A*, **156**, 1 (1978).
5. B. X. Mayer, E. Lorbeer, *J. Chromatogr. A*, **758**, 235 (1997).
6. J. Luong, R. Gras, W. Jennings, *J. Sep. Sci.*, **30**, 2480 (2007).
7. J.-M. D. Dimandja, G. C. Clouden, I. Colon, J.-F. Focant, W. V. Cabey, R. C. Parry, *J. Chromatogr. A*, **1019**, 261 (2003).
8. R. J. M. N. Dresen, H. J. W. Henderickx, van der Wal S., *J. Microcolumn Sep.*, **10**, 661 (1998).
9. *MXT® Capillary Columns Ideal for High Temperature GC Analysis* [http://blog.restek.com/wp-content/uploads/2014/05/GNTS1368A.pdf].
10. M. Inoue, Y. Saito, I. Ueta, T. Miura, H. Ohkita, K. Fujimura, K. Jinno, *Anal. Sci.*, **26**, 687 (2010).
11. G. Guiochon, C. I. Guillemin, *Quantitative Gas Chromatography for Laboratory Analyses and On-Line Process Control,* Elsevier, Amsterdam, The Netherlands (1988).

Audrius Zolumskis, Vida Vičkačkaitė, Augustė Kosinskaitė, Audrius Markevičius, Audrius Sadaunykas, Evaldas Naujalis, Simas Šakirzanovas

NAUJOS LABORATORIJOJE PAGAMINTOS NERŪDIJANČIO PLIENO KAPILIARINĖS DUJŲ CHROMATOGRAFINĖS KOLONĖLĖS SAVYBIŲ ĮVERTINIMAS

S a n t r a u k a

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.