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Green chromatography-carbon footprint of columns packed with core-shell materials

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Although, the acetonitrile and methanol are the most popular organic solvents employed in reversed-phase HPLC, it is important to minimizing the environmental impact of organic solvent usage during chromatographic analyses and the use of environmentally friendly solvents. Greener solvents i.e. ethanol are good organic modifier and environmentally preferable solvent that may replace toxic solvents in many RP-HPLC applications, with good chromatographic properties. At present study, critically evaluate of core-shell columns packed with different particle sizes (5.0, 2.6, 1.7, and 1.3 μm) was carried out to show the possibility of solvent consumption reduction and possibility to replace acetonitrile by ethanol in liquid chromatography. By comparing different columns packed with core-shell particles it was proven, that the organic solvent consumption may be reduced six times obtaining sufficient parameters of the analysis.

1. Introduction

High performance liquid chromatography (HPLC) is an analytical technique for separation, quantitation, and identification of chemical compounds. In recent years, improvements in both instrument and stationary phase technologies have greatly enhanced the performance of analytical laboratory in the industry and academia sections, leading to an excess of performance for many users. Nowadays, green analytical chemistry has developed to reduce or remove the use of environmentally hazardous organic solvents and reagents.[1-6] With growing awareness approximately, the wasting toxic chemical compounds on the worldwide "green" technologies developed throughout the world with extend beyond the direct implications for the environment affecting human health. Recent improvements in HPLC technology mean that greener, but less effective, solvents such as ethanol can be used without a significant loss in analytical capacity.

Different alternatives have been suggested to reduce the use and generation of harmful solvents in liquid chromatography, including micro flow HPLC,[6,7] or the use of stationary phases allowing a high water proportion in the mobile phases.[8] Traditional reversed-phase high performance liquid chromatography (RP-HPLC) thus becomes an attractive eco-separation technique using conventional stationary phases under simple and user-friendly experimental conditions.

In addition to the well-known principles of green chemistry,[9] three R (Reduce, Replace, Recycle) are commonly mentioned in connection with green analytical chemistry. In recent years, most of the efforts at greening analytical chromatography have focused on either replacement of hazardous solvents with green alternatives or in general, to reduce the amount of hazardous waste produced per unit of manufacture.[10] In addition, both from an environmental and an economic point of view, it is reasonable to remove the severe amounts of hazardous organic solvents for instance acetonitrile, methanol, etc. in the eluent of liquid chromatography by replacing with nontoxic components and "green" solvent i.e. ethanol.[11-14] Furthermore, pure water mobile phase used in reversed-phase chromatography is usually associated with phase collapse in case of chromatography on C18 phases.[15] Recently, some directions towards green analysis developed in chromatography are as follows:

- replacement of hazardous organic solvents with non-toxic and more green ones i.e. ethanol[16]
- reducing solvent hazardous usage in HPLC with decreasing the column dimension (e.g. column length, internal diameter, and/or particle size)
- operating at an elevated temperature[17]
- use of mobile phase additives such as cyclodextrins,[18] and surfactants to reduce the proportion of organic solvents.[19]
- by means of shorter chain alkyl groups of 1 and 8 carbon atoms bonded silica phases[20] and more polar reversed phases instead of long-chain alkyl groups such as on C18 and C30 bonded phases.[21]

– using core-shell column technology as an environmentally friendly stationary phase was developed and evaluated to achieve rapid separations with high efficiencies by using the shorter diffusion path of particles reduce axial dispersion of solutes.

In recent years, great *advanced techniques* in the new analysis methods to improving speed of chromatographic analysis are performed, which mostly based upon increased use of core-shell and smaller particle (d_p= 1.3 µm) stationary phases operating at higher back-pressures up to 1000 bar.[22-24] Theoretically, much higher flow rates can be used for smaller particles to improving the speed of separation in HPLC, because of the optimum linear velocity is inversely proportional to the particle size.[23]

$$V = \frac{\pi}{4} d_c^2 \times u \times t_R \quad (1)$$

where d_c is the column internal diameter, u is the linear velocity and t_R represent retention time.

In general, these advantages can be obtained irrespective of separation method or analyte structure, while some variation in the savings may be observed. It is evident from the UHPLC fast analysis technologies using smaller particles (d_p= 1.3 µm), higher pressures (Δp= 1000 bar), and shorter column lengths (L= 50 mm) with smaller diameters (ID= 2.1 mm) offer a greener alternative to conventional HPLC by organic solvent usage 0.61 ml for one run separation of test mixture.

The aim of this study was to determine the carbon footprints of core-shell stationary phases with different particle size and reduced column dimensions. The principal advantage of these materials is the use of available environmentally friendly solvents and reagents for liquid chromatography analyzing and extractions to follow the first principle of green chemistry that emphasizes waste prevention instead of remediation. In this work, we investigate the parameters involved in use of EtOH:H_2O mixture for greener analytical RP-HPLC applications by comparisons with mixtures of MeOH:H_2O, and ACN:H_2O mobile phases.

2. Experimental

2.1. Chemicals and materials

All standards, benzene, ethylbenzene, naphthalene, phenantrene, and pyren (concentration range 10–40 µg ml^{-1}) acetonitrile, methanol (HPLC grade) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethanol (HPLC grade) was purchased from J.T. Baker (Deventer, The Netherlands). The ultra-pure water used for mobile phase preparation was purified through a Millil-Q (Millipore, Bedford, MA). Four Kinetex-C18 columns (Phenomenex, Torrance, CA, USA) was tested. The physico-chemical properties of stationary phases are listed in Table 1.

Table 1. Physico-chemical properties of the Kinetex column given by the manufacturer

Parameter / Packing material	Total particle size (µm)	Column length (mm)	Column diameter (mm)	Pore size (Å)	Effective surface area (m^2/g)	Effective carbon load (%)	pH stability	Pressure stability (bar)
Kinetex C18	5.0	150	4.6	100	200	12	1.5–8.5	1000/600
Kinetex C18	2.6	150	4.6	100	200	12	1.5–8.5	1000/600
Kinetex XB-C18	1.7	100	2.1	100	200	10	1.5–8.5	1000
Kinetex C18	1.3	50	2.1	100	200	12	1.5–8.5	1000

2.2. Apparatus and methodology

The analyses were performed on a UHPLC Shimadzu Nexera chromatographic apparatus (Shimadzu Corporation, Kyoto, Japan) equipped with solvent delivery systems LC-30AD,, a SIL-30AC autosampler, CTO-20-AC column oven, DGU-20A3 on-line degasser, and SPD-M20A detector equipped with semi micro detection cell. The system control, data acquisition, and data evaluation were performed by Shimadzu "Lab-Solution" software (Shimadzu Corporation, Kyoto, Japan).

The isocratic mobile phase was prepared by on-line mixing in HPLC gradient grade organic solvents (ACN, MeOH or EtOH) and water.

The mobile phase composition for each column was optimized to get a resolution (R_s) value about 1.6 for the ethylbenzene and naphthalene pair. The flow rate was adjusted to obtain the highest efficiency for each column, by means of the van Deemter curve. Upon switching from a 4.6 mm ID column (5.0 and 2.6 µm of particle size) to 2.1 mm ID column (1.7 and 1.3 µm of particle size), in the case of acetonitrile and methanol solvents, 4.6 mm column is often operated at 1.0 ml min^{-1}, and the 2.1 mm column should be operated at 0.3 ml min^{-1}. However, in case of ethanol because of higher viscosity that causes high backpressure, flow rate for a columns with particles of 5.0, 2.6, and 1.7 (or 1.3 µm) was 1.0, 0.8, and 0.1 ml min^{-1}, respectively. A major advantage that evidently arises is the reduced solvent consumption with a factor of 4.8 without compromising separation. Switching from a 4.6 mm ID to a 2.1 mm column is no problem on state-of-the-art LC instrumentation, although analysis times can increase slightly due to the gradient delay caused by the volume of both the pump and injector. Reduction of the internal diameter is often accompanied with an increase in sensitivity. This is a direct consequence of the reduced dilution of the solutes in the mobile phase and the appearance of more concentrated bands at the detector.

The column temperature was set as 30 °C, and the injected volume were 1.0 and 0.1 µL for column with 4.6 mm and 2.1 mm internal diameter, respectively. Three parallel injections were performed at each flow rate and photometric UV detection at λ= 254 nm was applied. The peak profile data were acquired at frequencies of 100 Hz.

3. Results and discussion

3.1. Solvent replacement in RP-HPLC

The target of green analysis involves the use of less or non-toxic, renewable and green solvents,[25] which are an attractive feature, that ongoing measure also, include switching to materials with low organic solvent content and reducing consumption. The most common form of analytical separation technology performed in laboratories is RP-HPLC using a hydrophobic stationary phase and a mobile phase comprising acetonitrile with water containing additives to adjust pH and ionic strength. HPLC separations require organic solvents in high proportions generating a large volume of waste. Water is the most environmentally friendly solvent and in some case, the chromatographic separation can be achieved by use of pure water as mobile phase.[26]

Acetonitrile and methanol are used mainly as organic solvents in analytical HPLC, but they suffer from several drawbacks from the

viewpoint of green sustainable chemistry. Methanol has some disadvantages compared to acetonitrile in the HPLC applications. The column pressure drop will be increased because of the higher viscosity of methanol,[27] which gives lower efficiency and broad peaks. Since methanol has higher UV cut-off wavelength (λ= 205 nm) than acetonitrile (above λ= 195 nm), thus limits its usefulness in the low ultraviolet (UV) region.[28] The different analyte selectivities may be the most important factor that can occur, when switching solvent from acetonitrile to methanol or ethanol. Because of the toxicity of acetonitrile, these mixtures have to be treated as a hazardous waste. Thus, there is a growing interest in the replacement of non-toxic solvents, mainly alcohols, as an alternative to solve some specific chromatographic problems.[29,30] Replacement of acetonitrile due to its unique properties e.g. lower viscosity, high transparency in the UV region, and coupled with better chromatographic efficiencies is critical task. Therefore it's not easy to replace another solvent with acetonitrile. Hence, hazardous waste streams containing acetonitrile as chemical waste in the HPLC and can cause a great environmental pollution should be disposed. Since acetonitrile is much more expensive than other solvents in HPLC, due to its limited availability, it prices increased dramatically in 2009.[31]

In order to follow the first principle of green chemistry, greener mobile phases (i.e. methanol) or the less toxic solvent (i.e. ethanol as the sole organic solvent) are proposed in this work. Although changing to methanol has a lower pressure than ethanol but because of more amount of volume for separation, ethanol is preferred. This study clearly showed that ethanol performs reasonably well as an RP-HPLC solvent, and may be suitable for replacing acetonitrile in some instances. Use of the greener solvent i.e. ethanol may therefore be an option for some analytical laboratories, particularly those where the poorer elutropic strength and UV cut-off would not be a significant problem.

Table 2. Effect of different solvent and particle size on separation

Solvent / Particle size	ACN			MeOH			EtOH		
	organic solvent (%)	Run time (min)	Vol. organic solvent (ml)	organic solvent (%)	Run time (min)	Vol. organic solvent (ml)	organic solvent (%)	Run time (min)	Vol. organic solvent (ml)
5.0 µm	80	4.5	3.6	78.5	10.3	8.1	75	4.8	3.6
2.6 µm	82	3.8	3.1	82	6.5	5.3	78	4.5	2.8
1.7 µm	73.5	3.2	0.71	76	6.2	1.4	70.5	8.0	0.6
1.3 µm	62	3.3	0.61	70*	7*	1.47*	60	9.9	0.6

*due to lower efficiency in MeOH the resolution 1.6 was not achieved.

Although EtOH:H_2O mixture as an organic modifier has the same elution strength comparing with ACN:H_2O solutions at room temperature,[32] but due to higher viscosity, it is less favourable. As a result, the related pressure required for a typical chromatographic separation tends to be higher.[33]

The impact estimation of the effects of solvent change on retention volume of run time and percentage of organic solvent usage in similar conditions are summarized in Table 2. Because of changing acetonitrile solvent with ethanol by approximately at constant resolution of 1.6, retention factors of naphthalene for different column dimensions, namely d_p= 5.0, 2.6, 1.7, and 1.3 µm are 0.27, 0.23, 0.39, and 0.62, respectively. The proper retention volume, satisfied resolution, and separation can be accomplished with nontoxic solvent. Although disadvantage of this changing by higher pressure and reduce the lifetime of column make a limitation in flow rate profile. Therefore, with changing in solvent from acetonitrile to ethanol, in case of a d_p= 5.0 µm particle size, pressures increased from 85 to 288 bar. Although flow rate was the same for a d_p= 5.0 µm particle by the solvent changes from acetonitrile to ethanol, but for other particle size, was changed as follows: for d_p= 2.6 µm particle size is from 1.0 to 0.8 ml min^{-1} and for d_p= 1.7 and 1.3 µm, the flow rate is from 0.3 to 0.1 ml min^{-1}. In such condition, for a d_p= 2.6, 1.7, and 1.3 µm particle size, the pressure changes from 188, 268, and 395 bar to 548, 302 and 411 bar, respectively.

The most glaring change was the elution time of these compounds under limiting pressure conditions for the separation of a test mixture, when using acetonitrile as the organic modifier compared to methanol and ethanol as a solvent for column with 5.0 µm particle size, in constant resolution (R_s) value about 1.6 for the ethylbenzene and naphthalene pair (peaks 2 and 3) is illustrated in Figure 1.

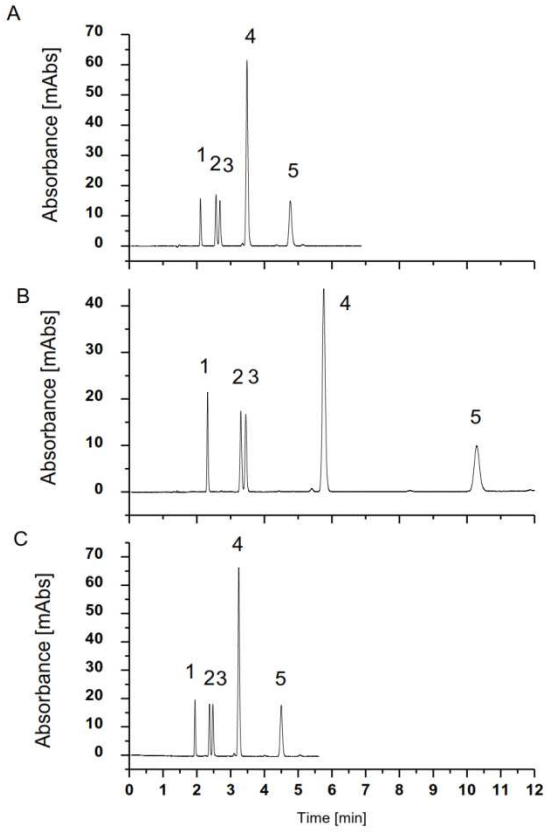

Fig. 1. Comparison of experimental chromatograms of test mixture separation (1-benzene,2-ethylbenzene,3-naphthalene, 4-phenantrene, 5-pyrene) on Kinetex C18 column (150 mm × 4.6 mm, 5 µm),

column temperature 30 °C: (A): 75/25$_{v/v}$ EtOH:H_2O, (B): 78.5/21.5$_{v/v}$ MeOH:H_2O, and (C): 80/20$_{v/v}$ ACN:H_2O

3.2. Solvent reduction by using fast and green separation

3.2.1. Fast separation by reduce the run time

The easiest way to reduce the solvent consumption in a separation process is to reduce the run time in the HPLC instrument. The mobile phase consumption (V_m) is given by the following expression:

$$V_m = F \times t_R \quad (2)$$

where F denote the volumetric flow rate and t_R represent run time of the separation.

By reducing in the column dimensions (i.e. column diameter, length and particle size), the quick and efficient separation will be achieved. However, pressure drop for column (50 × 2.1 mm, 1.3 µm) is much higher than the column (150 × 4.6 mm, 5.0 µm) even though lower flow rate was used. The column length, L, required for a given separation is related to the particle size:[34]

$$L = 2N_{req} \times d_p \quad (3)$$

where N_{req} is the required column efficiency for a given separation and d_p denotes the particle size of the packing material.

It can be seen that, while columns provide about similar separation, the separation speed for the shorter column (50 × 2.1 mm, 1.3 µm particles) is nearly two times faster than the longer column (150 × 4.6 mm, 5 µm particles) simply because the former is three times shorter. Consequently, by reducing column length and particle diameter size, a solvent saving of approximately six-fold can be achieved (see Table 2).

3.2.2. Green separation with core-shell column technologies

A number of approaches exist to reduce the solvent use for HPLC. Changing column dimensions and/or particle size, and reducing column equilibration time can be altered to significant solvent savings by achieving more efficient, sensitive, and faster analyses.[35] The core-shell columns implement faster separation and offered lower back-pressure compared to traditional columns typically employed in UHPLC. Thus, the waste generated is typically lower than traditional columns.

During the study scale-down column dimensions was performed. The initial analysis was conducted on a standard analytical scale 150 mm × 4.6 mm ID × 5.0 µm HPLC column. To speed the analysis, the internal diameter of the column to ID= 2.1 mm was decreased and reduced the particle size to d_p= 1.3 µm as well as column length (Table 2). By reduction of column diameter the acetonitrile consumption can be reduced approximately 34% and the run time was shortened by 37%. Decreasing the column length from 150 mm to 100 mm and 50 mm decrease the ACN consumption from 3.1 ml to 0.71 ml and 0.61 ml, respectively. Improved resolution and a decrease in run time and equilibration times were additional benefits. All data for MeOH, ACN and EtOH are summarized in Table 2. In Figure 2 an example of a greener separation, using different core-shell columns are presented.

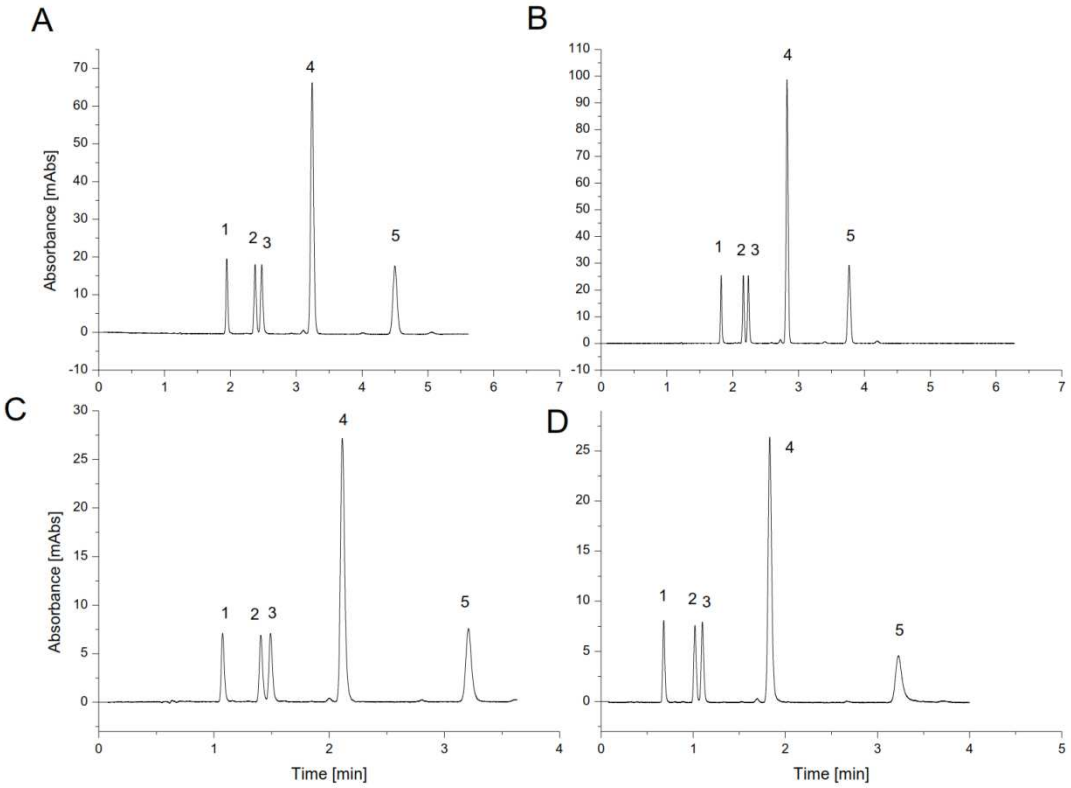

Fig. 2. Comparison of separations performed on different column dimensions, (A) 150 mm × 4.6 mm, 5 µm flow = 1.0 ml min^{-1}, 80/20$_{v/v}$ ACN:H_2O, (B) 150 mm × 4.6 mm, 2.6 µm flow = 1.0 ml min^{-1}, 82/18$_{v/v}$ ACN:H_2O (***C***) 100 mm × 2.1 mm, 1.7 µm flow = 0.3 ml min^{-1}, 73.5/26.5$_{v/v}$ ACN:H_2O , (D) 50 mm × 2.1 mm, 1.3 µm flow = 0.3 ml min^{-1}, 62/38$_{v/v}$ ACN:H_2O for test mixture (1-benzene, 2-ethylbenzene, 3-naphthalene, 4-phenantrene, 5-pyrene) on Kinetex C18 columns, column temperature of 30 °C.

To minimize solvent consumption a smaller column diameter can be used with d_p= 1.3 µm particles to achieve higher optimum linear velocity. Reducing the internal diameter of HPLC columns can intensely lead to reduce hazardous waste solvent generations are presented in the following equation. A flow rate of 0.3 ml min^{-1} can therefore be used for a column packed with d_p= 1.7 µm and 1.3 µm particles to achieve the same linear velocity about 2.5, separation speed and column efficiency, affording about six-fold additional solvent savings (see Fig. 2). As you can see in Table 2, a reduction in solvent consumption and waste generation is observed only with minor differences in selectivity observed between the separations by comparison of the volume of mobile phase for elution time in an HPLC column.

As seen in Fig. 3, ACN can be replaced by EtOH. Such change allows to perform sufficient separation of test mixture. Unfortunately, due to the higher viscosity of EtOH/water mixtures, the flow rate has to be reduced for columns with lower particle size. As a result, the time of the separation increases in the comparison with the ACN as an organic modifier. It has to be mention that the volume of the EtOH became slightly lower than volume of ACN used. The results confirm, that liquid chromatography may be carried out with green solvent, such as ethanol.

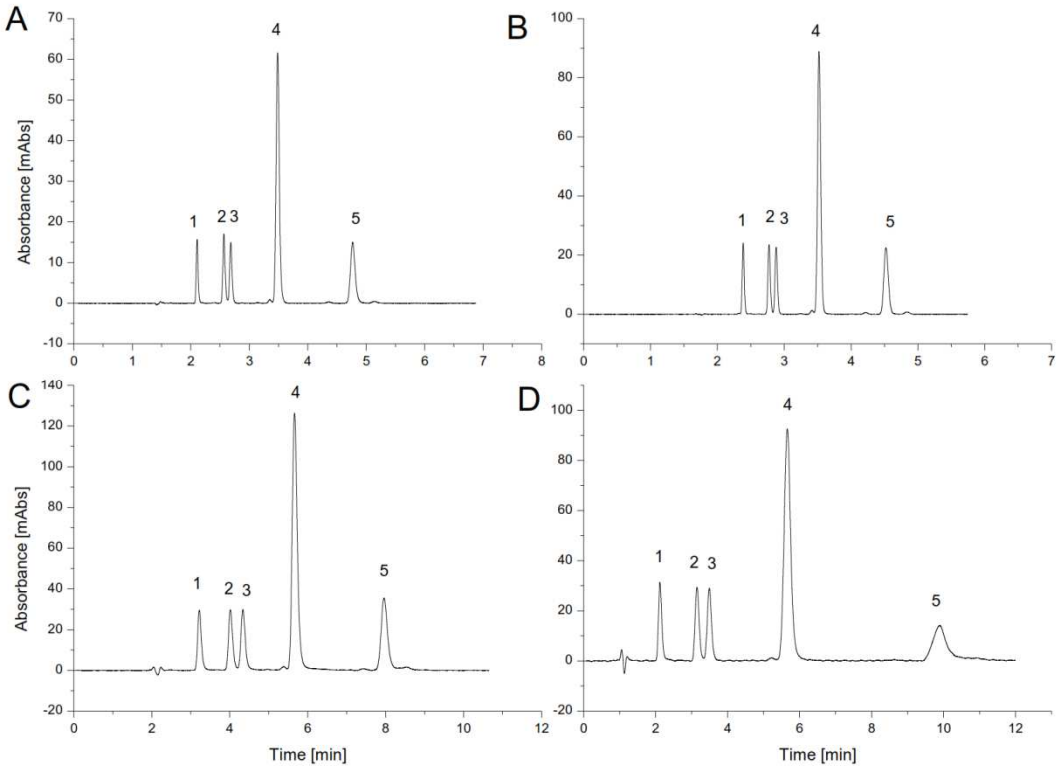

Fig. 3. Comparison of separations performed on different column dimensions, (A) 150 mm × 4.6 mm, 5 µm flow = 1.0 ml min^{-1}, $75/25_{v/v}$ EtOH:H_2O, (B) 150 mm × 4.6 mm, 2.6 µm flow = 0.8 ml min^{-1}, $78/22_{v/v}$EtOH:H_2O (***C***) 100 mm × 2.1 mm, 1.7 µm flow = 0.1 ml min^{-1}, $70.5/29.5_{v/v}$EtOH:H_2O , (D) 50 mm × 2.1 mm, 1.3 µm flow = 0.1 ml min^{-1}, $60/40_{v/v}$EtOH:H_2O for test mixture (1-benzene, 2-ethylbenzene, 3-naphthalene, 4-phenantrene, 5-pyrene) on Kinetex C18 columns, column temperature of 30 ^{o}C.

Conclusions

This work confirms by using new core-shell Kinetex C18 column, replacement of acetonitrile by ethanol in mobile phase has very similar performance in RP-HPLC applications. In line with reducing the particle size (from 5.0 to 1.3 µm) in combination with shortening the column length (from 150 to 50 mm) and also decreasing its internal diameter (from 4.6 to 2.1 mm) provide a rapid throughput design, lower solvent consumption, low detection level, and high reproducibility. Hence, by changing core-shell particle size from 5.0 to 1.3 µm, the column void volume for the acetonitrile and ethanol solvents, are decreased from 1.95 to 0.2 ml and 2.11 to 0.21 ml, respectively. Under such conditions, the possibilities offered by this column technology are efficient, and the 1.3 µm core-shell particles are particularly attractive and exclusively be used on powerful state-of-the-art UHPLC system possessing an upper pressure limit approximately 1000 bar. The separation times (3.3 min) and reduction in acetonitrile consumption (0.61 ml) can be up to an order of magnitude compared to longer columns

packed with 5.0 µm (3.6 ml). It was proven that toxic solvent used in liquid chromatography may be replaced by ethanol.

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Notes and references

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