European Journal of Chemistry

View Journal Online
View Article Online

Simultaneous determination of citalopram, paroxetine, fluoxetine, and sertraline by high-temperature liquid chromatography

Sema Akay * and Berkant Kayan

Department of Chemistry, Faculty of Arts and Science, Aksaray University, Aksaray, TR 68100, Turkey
e-mail: sema.akay7@gmail.com (S.A.), berkantkayan@gmail.com (B.K.)

** Corresponding author at: Department of Chemistry, Faculty of Arts and Science, Aksaray University, Aksaray, TR 68100, Turkey.*
Tel: +90.382.2882166 Fax: +90.382.2882145 e-mail: sema.akay7@gmail.com (S. Akay).

RESEARCH ARTICLE

10.5155/eurjchem.9.3.182-188.1738

Received: 25 May 2018
Received in revised form: 27 June 2018
Accepted: 29 June 2018
Published online: 30 September 2018
Printed: 30 September 2018

KEYWORDS

Column
Green chemistry
Chromatography
High-temperature
Antidepressant agent
Selective serotonin reuptake inhibitor

ABSTRACT

A lack of serotonin in the brain is associated with depression. Selective serotonin reuptake inhibitors (SSRIs) are widely used to help treat depression and associated symptoms. A method has been developed for the simultaneous determination of SSRIs by high-temperature liquid chromatography (HTLC). Citalopram, paroxetine, fluoxetine, and sertraline compounds, which are widely used as antidepressant active agents, have been chosen as SSRIs. The separation of the SSRIs have been carried out by using four different column types, including XTerra MS C18, Zorbax SB-Phenyl, Alltima C18 and Phenyl Hypersil columns, and their chromatographic performances have been evaluated. The best separation has been obtained on the Zorbax SB-Phenyl column (150 mm × 4.6 mm, 5 µm) among the four different columns studied. The separation temperature and the composition of mobile phase were examined for the optimization of chromatographic separation. Chromatographic separation of SSRIs has been carried out at temperatures ranging from 100 to 200 °C with variable flow rates (0.5-1.5 mL/min). Water:acetonitrile:acetic acid mixtures containing with 10 or 20% acetonitrile and 2% acetic acid have been used as mobile phase. The best separation was observed at volume ratio of 78:20:2 (water:acetonitrile:acetic acid) at elevated temperature on the Zorbax SB-Phenyl column. The wavelength of UV detector was set at 254 nm. All four analytes were eluted within 8 min at 200 °C. At the end of working, it was observed that the retention times of all four analytes decreased with increasing temperature and was stated that the temperature was an effective parameter for chromatographic separation. Furthermore, the relationship between retention factor and separation temperature was examined using Van't Hoff plots and the results demonstrated with correlation coefficient greater than 0.91 on Zorbax SB Phenyl column. Consequently, the proposed HTLC method for separation and analysis of SSRIs may be used as a green alternative technique.

Cite this: *Eur. J. Chem.* **2018**, *9(3)*, 182-188 **Journal website**: www.eurjchem.com

1. Introduction

Over the last decade, green chromatography techniques have gained increasing attention and acceptance from researchers both academia and industry. High-temperature liquid chromatography is one of these techniques [1-9]. HTLC exists in a temperature beyond room temperature (ca. 40 C) and below supercritical temperatures [10]. This technique deals with liquid chromatography separations performed at temperatures typically within a range from 40 to 200 °C using organic solvent-water mixtures as the mobile phase [3,5,8].

Because of the long retention times in conventional reversed phase-high performance liquid chromatography (RP-HPLC) separations, the solvent consumption is proportionately high [11]. HTLC has several advantages when compared with the conventional HPLC. Major advantages of HTLC include increased efficiency, shortened analysis time, and reduction in mobile phase used an organic solvent. Due to the reduction of the dielectric constant of liquids at elevated temperature could be reduce the ratio of organic solvents in the mobile phase, as long as the temperature is raised appropriately [12,13]. Moreover, the accompanying decrease in mobile phase viscosity provides a lowering of column back pressure, allowing faster separations [13]. The decrease of viscosity at increasing temperatures allows the use of higher flow rates, as well [11]. Consequently, the increase in temperature increases diffusivity of the analyte, resulting in improved column efficiency. All of these positive effects have ensured an increased interest in conducting liquid chromatography separations at elevated temperature [13,14].

High-temperature LC (HTLC) is a promising eco-friendly technique for conventional RP-HPLC. However, it is not widely applied in analysis of pharmaceuticals due to limited availability of thermally stable stationary phases at elevated temperature [15]. But, in a series of papers, Yang *et al.* [3,7,16-18] concluded that the results obtained by a long-term stability study are quite encouraging. With the help of newly developed stationary phases that are thermally more stable and reliable, liquid chromatographic separations may be

http://dx.doi.org/10.5155/eurjchem.9.3.182-188.1738

Table 1. Description of columns tested.

Column name	Length (mm)	I.D. (mm)	Particle size (µm)	Pore size (Å)	Base material
X-Terra MS C18	100	4.6	3.5	125	Hybrid silica
Zorbax-SB C18	150	4.6	5	80	Porous silica
Alltima C18	150	4.6	5	100	Spherical silica
Phenyl Hypersil	150	4.6	5	120	Porous silica

carried out at elevated temperatures without decomposition of the packing materials.

Depression is a common, life-disrupting, potentially lethal illness that can affect all ages [19]. A lack of serotonin in the brain is associated with depression [20,21] that is why drugs called selective serotonin reuptake inhibitors (SSRIs) are widely used to help treat depression [19]. The SSRIs are also effectively used in anxiety, obsessive-compulsive disorder, panic disorder and social phobia [22], and they have occasionally been prescribed for pre-menstrual dysphoric disorder and in the treatment of eating disorders, as well [23]. The SSRIs have a different, and generally better tolerated, adverse effect profile when compared to the other antidepressants with approximately equivalent efficacy [19,24,25]. The antidepressants such as citalopram, paroxetine, fluoxetine, fluvoxamine and sertraline belong to the class of SSRIs [19].

Some studies have been published for the simultaneous determination of several compounds of the SSRI by analytical methods. Almost all assays are based either on separation of SSRIs by high-performance liquid chromatography (HPLC) or gas chromatography (GC) [26,27] and detection by various detectors such as ultraviolet [28], diode array detection [29], fluorescence [30], electrochemical detector, mass spectrometry, nitrogen-phosphorus detector [31,32]. However, no study has been conducted to determine the four compounds of the SSRI simultaneously by high-temperature liquid chromatography.

In the present study, was investigated the potential application of the HTLC technique for separation of SSRIs. The SSRIs such as citalopram, paroxetine, fluoxetine, and sertraline, which are pharmacologically and environmentally prescriptive and selected as model molecules, have been used. The separation temperature and the composition of mobile phase were examined for the optimization of chromatographic separation conditions. Furthermore, the influence of temperature on retention factor was investigated using Van't Hoff plots.

2. Experimental

2.1. Chemicals and equipments

Citalopram hydrobromide (CIT), paroxetine hydrochloride (PRX), fluoxetine hydrochloride (FLU), and sertraline hydrochloride (SRT) were purchased from Sigma Aldrich Chemical (Steinheim, Germany). HPLC grade acetonitrile and acetic acid were acquired from Merck Chemical (Istanbul, Turkey). Ultrapure water (18.2 MΩ×cm) was obtained from a MilliPore Milli-Q-Gradient water purification system (Billerica, MA, USA).

XTerra MS C18 column was purchased from Waters Corporation (Milford, USA), and Zorbax SB-Phenyl column was acquired from Agilent Technologies (Santa Clara, CA, USA). Alltima C18 column was obtained from Grace Materials Technologies (Columbia, USA), and Phenyl Hypersil column was purchased from Thermo Fisher Scientific (Waltham, USA).

A Perkin-Elmer Flexar LC system (Waltham, USA) with an ISCO 260-D syringe pump (Teledyne ISCO, Lincoln, NE, USA), Teknosem column heating unit (Istanbul, Turkey), a Peltier cooling unit (Teknosem, Istanbul, Turkey), Flexar LC injector with a 10 µL loop, a back pressure regulator and the Flexar UV detector was used.

2.2. Chromatographic conditions

An ISCO 260-D syringe pump and a Perkin-Elmer Flexar LC system were combined and converted into a high-temperature liquid chromatograph. The detailed procedures are described in our previous study [18]. Chromatographic separations were achieved on the determined four commercial columns at different temperatures (100, 150, 175, and 200 °C). Water: acetonitrile:acetic acid mixtures containing with 10 or 20% acetonitrile and 2% acetic acid have been used as mobile phase in separations. Acetic acid was used to control the pH of the mobile phase. Analyte concentrations in test mixture were ranging 20-40 mg/L, and they were freshly prepared using ultra-pure water as the solvent. Chromatographic separations were carried out at flow rates ranging from 0.5 to 1.5 mL/min. The injection volume was 10 µL and the wavelength of detector was set at 254 nm. Data acquisition and analysis were performed with the Perkin-Elmer Chromera chromatography data system. The obtained chromatograms were investigated by taking into account the qualitative separation of solutes.

3. Results and discussion

3.1. Column selection and chromatographic separations

The performance of the chromatographic system is determined by the separation carried out in the column, namely the selection and use of the appropriate column filling material. Most of columns used in reversed-phase liquid chromatography contain silica-based phases such as C8, C18. Silica-based RP columns have been widely employed in terms of the popularity and good efficiency.

In this study were chosen silica-based different column types such as XTerra MS C18, Zorbax SB-Phenyl, Alltima C18 and Phenyl Hypersil, because literature search shows that these stationary phases are relatively stable at high temperatures [3,7,9]. The description of all four columns is given in Table 1.

3.1.1. Zorbax SB-phenyl column

The separation of SSRIs was first investigated on the Zorbax SB-Phenyl column. Chromatographic separations on this column were carried out at temperatures ranging from 100 to 200 °C with ranging 1.0-1.3 mL/min flow rates. Firstly, water-organic solvent mixtures (water:acetonitrile:acetic acid, 93:5:2, *v*:*v*:*v*) have been used as mobile phase in separation. Despite the use of high temperatures, good separation was not achieved and the retention times were relatively long. Separations of the same test mixture were also performed using a stronger mobile phase with 20% acetonitrile and the obtained chromatograms are shown in Figure 1. To limit the length of this paper, only chromatograms obtained at 150 and 175 °C are used.

As shown in Figure 1, that all four analytes are well separated and no extra peaks are observed. The retention times of all four analytes decreased at elevated temperatures with adding organic solvent. In addition, it can clearly be seen in Table 2.

Table 2. HTLC data obtained on four different columns depending on the amount of acetonitrile in the mobile phase with the temperature.

Temperature (°C)	Flow rate (mL/min)	Compounds	Retention time	Retention factor	Tailing factor	Peak width
Zorbax SB Phenyl (20 % Acetonitrile)						
100	1.0	Citalopram	12.16	36	0.94	1.39
		Paroxetine	13.48	49	0.93	1.35
		Fluoxetine	17.84	67	0.84	1.58
		Sertraline	21.60	75	0.79	1.67
150	1.0	Citalopram	7.42	29	0.99	1.30
		Paroxetine	9.01	36	0.95	1.24
		Fluoxetine	12.04	48	0.93	1.49
		Sertraline	14.61	58	0.89	1.56
175	1.2	Citalopram	4.58	21	1.02	1.17
		Paroxetine	6.15	24	0.98	1.11
		Fluoxetine	8.62	33	0.97	1.40
		Sertraline	9.96	39	0.95	1.45
200	1.3	Citalopram	3.01	17	1.05	1.03
		Paroxetine	4.12	22	1.02	1.07
		Fluoxetine	6.95	27	0.99	1.37
		Sertraline	7.87	32	0.97	1.39
XTerra MS C18 (10 % Acetonitrile)						
100	0.5	Citalopram	16.34	64	0.80	2.15
		Paroxetine	29.57	99	0.69	2.36
		Sertraline	52.12	157	0.51	2.67
		Fluoxetine	52.58	159	0.52	2.69
150	0.8	Citalopram	6.47	25	0.93	1.89
		Paroxetine	10.12	40	0.89	2.22
		Sertraline	15.81	61	0.91	2.28
		Fluoxetine	15.96	64	0.89	2.29
175	0.9	Citalopram	4.10	15	0.97	1.81
		Paroxetine	5.87	23	0.92	2.14
		Sertraline	9.65	38	0.95	2.17
		Fluoxetine	9.73	40	0.92	2.18
200	1.0	Citalopram	2.48	9	1.00	1.76
		Paroxetine	3.90	11	0.98	2.05
		Sertraline	6.01	27	0.99	2.11
		Fluoxetine	6.04	27	0.99	2.12
Alltima C18 (20 % Acetonitrile)						
100	1.0	Citalopram	12.58	40	0.80	2.24
		Paroxetine	15.94	59	0.75	2.47
		Fluoxetine	24.01	97	0.71	2.56
		Sertraline	26.29	101	0.73	2.59
150	1.1	Citalopram	7.02	27	0.89	2.13
		Paroxetine	9.88	38	0.84	2.33
		Fluoxetine	18.72	71	0.81	2.49
		Sertraline	18.93	72	0.80	2.51
175	1.1	Citalopram	5.21	20	0.92	1.94
		Paroxetine	7.85	31	0.87	2.19
		Fluoxetine	13.87	54	0.92	2.42
		Sertraline	14.02	56	0.91	2.43
200	1.2	Citalopram	3.98	14	0.97	1.85
		Paroxetine	5.66	23	0.90	2.07
		Fluoxetine	9.70	38	0.99	2.31
		Sertraline	9.81	39	0.97	2.31
Phenyl Hypersil (10 % Acetonitrile)						
100	1.0	Citalopram	72.10	251	0.84	6.02
		Paroxetine	95.21	339	0.78	7.10
		Fluoxetine	-	-	-	-
		Sertraline	-	-	-	-
150	1.3	Citalopram	49.18	193	0.96	4.88
		Paroxetine	70.03	278	0.90	5.92
		Fluoxetine	94.60	357	0.89	7.21
		Sertraline	-	-	-	-
175	1.5	Citalopram	33.37	134	1.00	4.35
		Paroxetine	51.76	203	0.98	5.47
		Fluoxetine	76.89	295	0.96	6.54
		Sertraline	-	-	-	-
200	1.5	Citalopram	18.95	72	1.03	3.92
		Paroxetine	32.35	135	1.00	5.09
		Fluoxetine	58.11	248	0.98	6.29
		Sertraline	-	-	-	-

3.1.2. Separations on XTerra MS C18

The separation of SSRIs was investigated on the XTerra MS C18 column at temperatures ranging from 100 to 200 °C. The separations were obtained with a mobile phase consisting of 10% acetonitrile (water:acetonitrile:acetic acid, 88:10:2, *v*:*v*:*v*) at flow rates ranging from 0.5 to 1.0 mL/min. During the experiments it was stated that the separation with increasing amount of organic solvent performed better, and the elution accelerated with increasing temperature. Figure 2 has shown the chromatograms obtained on this column at different temperatures and varied flow rates.

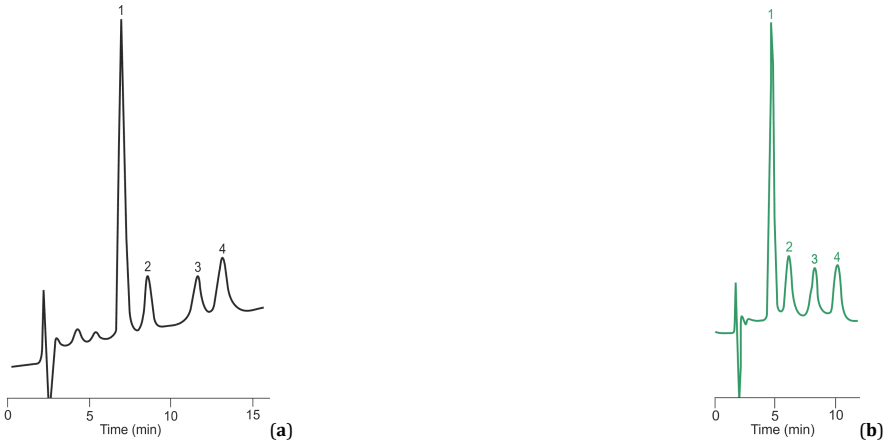

Figure 1. Separation of the SSRIs with HTLC on Zorbax SB Phenyl column at (**a**) 150 °C and (**b**) 175 °C. Peak identification: 1, citalopram; 2, paroxetine; 3, fluoxetine and 4, sertraline.

Figure 2. Separation of the SSRIs with HTLC on XTerra MS C18 column at (**a**) 150 °C and (**b**) 175 °C. Peak identification: 1, citalopram; 2, paroxetine; 3, sertraline and 4, fluoxetine.

As investigated is Figure 2, no extra peak were observed but, fluoxetine and sertraline were co-elueted in separation, and then citalopram and paroxetine were also co-elueted at 200°C. Thus, good separation was not obtained. It was concluded that this may be due to the fact that XTerra MS C18 column has different properties such as the column length and particle size than the other columns studied. However, it was stated that the retention times of all four analytes decreased with increasing temperature and then the shortest retention time among all columns was achieved on the XTerra MS C18 column at 200 °C with a time of less than 7 minutes.

3.1.3. Separations on Alltima C18

The separation of SSRIs was performed on the Alltima C18 column at temperatures ranging from 100 to 200 °C with ranging 1.0-1.2 mL/min flow rates. The separations were obtained with a mobile phase consisting of 20% acetonitrile (water:acetonitrile:acetic acid, 78:20:2, *v*:*v*:*v*). Some of the chromatograms obtained by Alltima C18 column is shown in Figure 3.

The retention times of all four analytes decreased at elevated temperature, but fluoxetine and sertraline were co-elueted as in XTerra column, and so good separation was not achived on this column, as well. In order to make a better separation, it was stated that the composition of mobile phase should be changed, that is, the ratio of the organic solvent should be increased. However, it was concluded that even if the ratio of organic solvent is increased, citalopram and paroxetine peaks will be co-eluted with increasing temperature, so that an effective separation cannot be made.

3.1.4. Separations on phenyl hypersil

The separation of SSRIs was finally investigated on the Phenyl Hypersil column (Figure 4). The separations were obtained with a mobile phase consisting of 10% acetonitrile (water:acetonitrile:acetic acid, 88:10:2, *v*:*v*:*v*) at temperatures ranging from 100 to 200 °C with ranging 1.0-1.5 mL/min flow rates.

As shown in Figure 4, all three analytes were eluted within 95 min in 150 °C, and sertraline peak was not detected in the seperations. It was stated that the reason is due to the structure of the stationary phase and its interaction with the analytes. Despite the use of high temperatures and high flow rates, good separations were not achieved and the retention times were relatively long on the Phenyl Hypersil column. In addition, the solvent consumption was proportionately high due to the long retention times in separations. Thus, good separation was not obtained on this column.

3.2. Influence of temperature on retention

A good strategy for greening LC methods is to minimize the consumption of organic solvents is to speed up the analysis by elevating the mobile phase temperature [15].

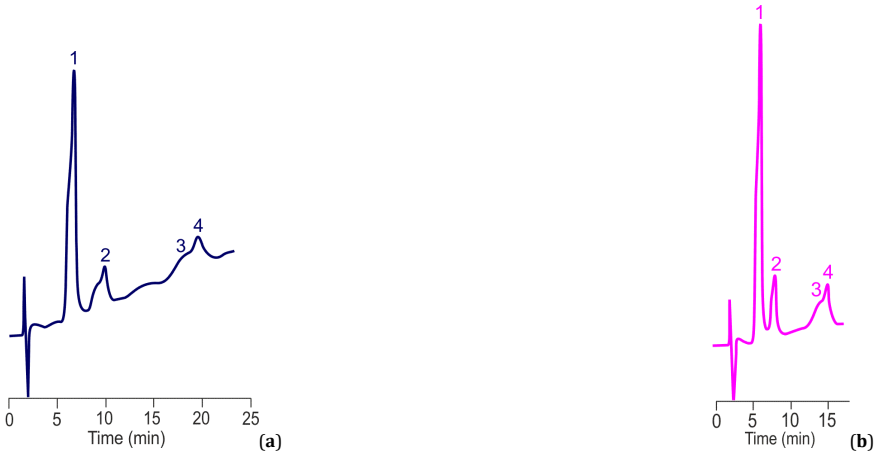

Figure 3. Separation of the SSRIs with HTLC on Alltima C18 column at (**a**) 150 °C and (**b**) 175 °C. Peak identification: 1, citalopram; 2, paroxetine; 3, fluoxetine and 4, sertraline.

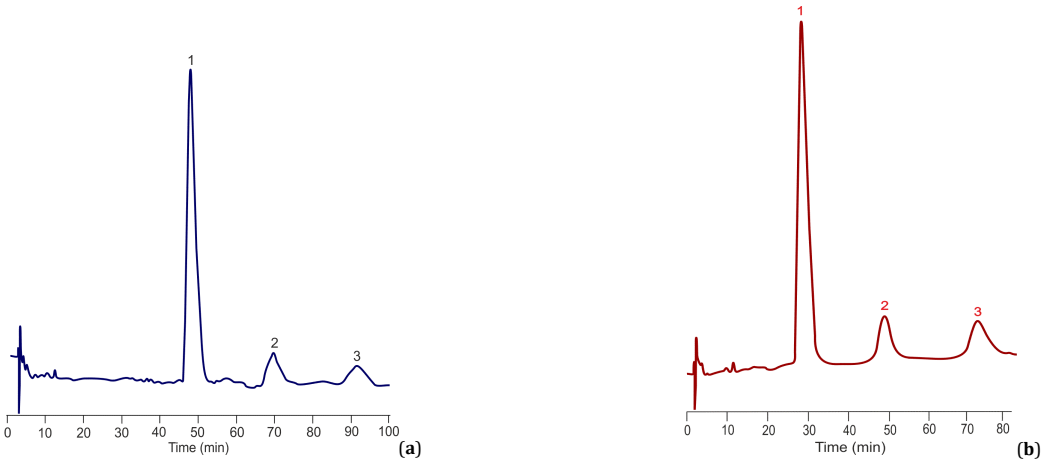

Figure 4. Separation of the SSRIs with HTLC on Phenyl Hypersil column at (**a**) 150 °C and (**b**) 175 °C. Peak identification: 1, citalopram; 2, paroxetine; 3, fluoxetine.

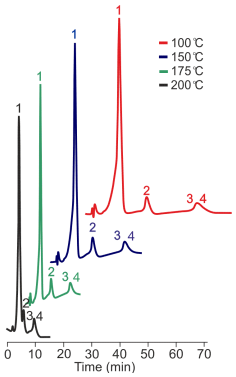

Figure 5. Influence of temperature on retention times of SSRI in XTerra MS C18 column. Peak identification: 1, citalopram; 2, paroxetine; 3, sertraline and 4, fluoxetine.

Because temperature is a strong variable that affects selectivity and efficiency. Elevated temperature can significantly reduce the retention times of analytes (Figure 5). In addition, Table 2 summarizes chromatographic data such as retention times (min), retention factors, tailing factors, and peak width (min) for HTLC separations at working temperatures. Along with the increased flow rate, the peak widths of the compounds were reduced as well as the retention times. Besides the mobile phase additives such as acetic acid, the high temperature has also improved the peak symmetries of the compounds.

The relationship between separation temperature and retention factor was examined using van't Hoff plots. The result demonstrated linear correlation between ln(k') and 1/T for separations on Zorbax SB Phenyl column with correlation coefficient greater than 0.91 (Figure 6).

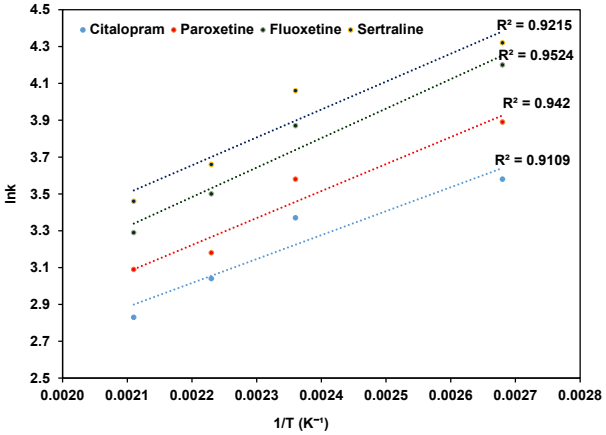

Figure 6. Van't Hoff Plots for separations on Zorbax SB Phenyl column.

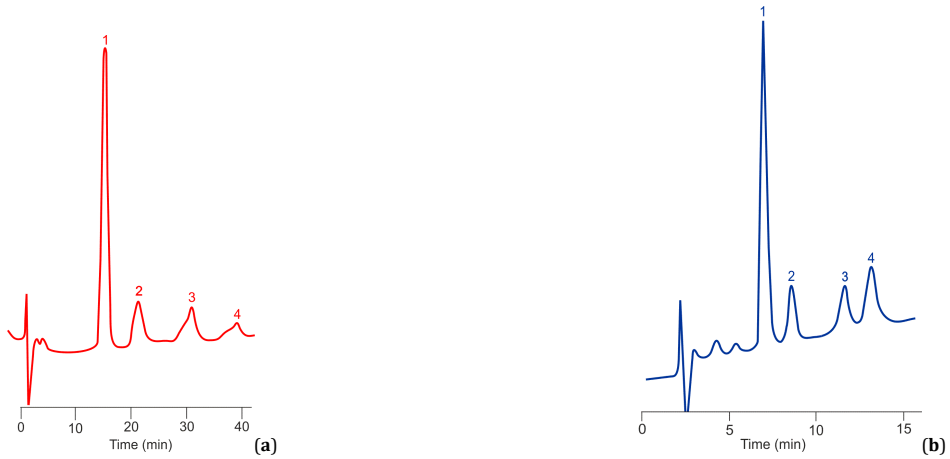

Figure 7. Influence of composition of mobile phase on HTLC separations in Zorbax SB Phenyl column. (**a**) 10 % Acetonitrile at 150 °C (**b**) 20 % Acetonitrile at 150 °C. Peak identification: 1, citalopram; 2, paroxetine; 3, fluoxetine and 4, sertraline.

In addition, the standard enthalpy (ΔH^o) values for SSRIs were calculated from the slope of the Van't Hoff plots and were obtained the values for all solutes are negative. The standard enthalpy values calculated for citalopram, paroxetine, fluoxetine and sertraline are ΔH_{CIT}^o= -10.81 kJ/mol; ΔH_{PRX}^o= -12.18 kJ/mol; ΔH_{FLU}^o= -13.30 kJ/mol; ΔH_{SRT}^o= -12.60 kJ/mol, respectively. It was stated that solute transfer is an exothermic process, and it is energetically more suitable for the solutes to remain in the stationary phase.

Due to the viscosity of the mobile phase is significantly decreased with increasing temperatures, the obtained low back-pressure allows to be employed much higher flow rates [3]. Thus, the increased flow rate greatly decreases the retention time, and shortens the exposure of the analyte to high temperatures. In addition, analyte degradation is not observed, and stationary phases are not much damaged, as well. Therefore, the advantages of studying at elevated temperatures such as reduced solvent consumption, shorten analysis time, and efficient separation allow HTLC to be used as a green tool for routine analysis.

3.3. Influence of composition of mobile phase on separation

The composition of mobile phase is another important parameter in separation. Most of the HTLC studies [1,13,18,33] used of water-organic solvent mixtures as mobile phases. This is because the elution strength of pure heated water is not strong enough for analysis most of the non-polar analytes in commercially available reversed-phase columns [33].

Considering that water acts as an organic solvent during the separations carried out at high temperatures [3,7,34,35], the ratio of modifier used in the mobile phase was kept constant at maximum 20% (Figure 7). In addition, it is believed that a high ratio of organic solvent is disadvantageous both in terms of chemical consumption and environment.

As shown in Figure 7, these significant improves in separation efficiency with modifier in mobile phase can clearly be seen as an added benefit to performing the separations at elevated temperatures. Consequently, at elevated temperature the use of acetonitrile-water mixture as a mobile phase can be an efficient method to separate SSRIs with the benefit of consuming little acetonitrile than conventional separation methods.

4. Conclusion

Chromatographic analysis of citalopram, paroxetine, fluoxetine, and sertraline were carried out on four commercial columns using HTLC. Due to their better stability at higher temperatures, these columns were chosen in this work. The best separations were achieved on Zorbax SB-Phenyl column. No extra peaks were observed in columns, namely analyte degradation is not observed and also these stationary phases

are stable under high-temperature liquid chromatography conditions. Although HTLC separation of SSRIs samples were carried out in this work, industrial application of the HTLC methods developed in this study will require further validation studies. The separation temperature is the most important parameter for the optimization of chromatographic separation conditions in this study. In that, the relationship between retention factor and separation temperature was examined using van't Hoff. It was observed that the retention times of all four solutes decreased with increasing temperature. Besides, the ratio of organic solvent in mobile phase is considerably reduced with increasing temperature. Thus, the results obtained in this study indicated that using acetonitrile–water mixtures at elevated temperatures as an alternative to the conventional RP-HPLC mobile phases can be reduce the ratio of acetonitrile used to achieve effective separation. On the other hand, it has been noted in the study that the mobile phase composition, i.e. the acetonitrile ratio, is the second effective parameter for separation. The best separation was observed at volume ratio of 20% acetonitrile at elevated temperature with the Zorbax SB-Phenyl column. Namely, the power of elution of the pure water was increased with acetonitrile in mobile phase. It was stated that the separation with increasing amount of organic solvent performed better. Therefore, the retention time significantly decreased with increasing temperature combined with a stronger modifier. It was stated the advantages of studying at elevated temperatures such as shorten analysis time and efficient separation allow HTLC usage. Consequently, the data of study demonstrated that HTLC may be used as a green alternative technique for separation and analysis of SSRIs.

Acknowledgments

The authors gratefully thank the Scientific Research Project Unit (BAP) of Aksaray University (2016/038) for the financial support of this work.

Disclosure statement

Conflict of interests: The authors declare that they have no conflict of interest.
Author contributions: All authors contributed equally to this work.
Ethical approval: All ethical guidelines have been adhered.
Sample availability: Samples of the compounds are available from the author.

ORCID

Sema Akay
http://orcid.org/0000-0001-8887-6653

Berkant Kayan
http://orcid.org/0000-0002-9892-5284

References

[1]. Greibrokk, T.; Andersen, T. *J. Chromatogr. A* **2003**, *1000*, 743-755.
[2]. Teutenberg, T.; Goetze, H. J.; Tuerk, J.; Ploeger, J.; Kiffmeyer, T. K.; Schmidt, K. G.; Kohorst, W.; Rohe, T.; Jansen, H. D.; Weber, H. *J. Chromatogr. A* **2006**, *1114*, 89-96.
[3]. Yang, Y. *J. Sep. Sci.* **2007**, *30*, 1131-1140.
[4]. Smith, R. M. *J. Chromatogr. A* **2008**, *1184*, 441-455.
[5]. Heinisch, S.; Rocca, J. L. *J. Chromatogr. A* **2009**, *1216*, 642-658.
[6]. Welch, C. J.; Wu, N.; Biba, M.; Hartman, R.; Brkovic, T.; Gong, X.; Helmy, R.; Schafer, W.; Cuff, J.; Pirzada, Z.; Zhou, L. *Trends Anal. Chem.* **2010**, *29*, 667-680.
[7]. Yang, Y.; Kapalavavi, B. Subcritical Water Chromatography-An Economical and Green Separation Technique, John Wiley, 2011.
[8]. Kapalavavi, B.; Marple, R.; Gamsky, C.; Yang, Y. *Int. J. Cosmet. Sci.* **2012**, *34*, 169-175.
[9]. Kayan, B.; Akay, S.; Yang, Y. *J. Chromatogr. Sci.* **2016**, *54*, 1187-1192.
[10]. Reid, R. C.; Prausnitz, J. M.; Sherwood, T. K. The Properties of Gases and Liquids, McGraw-Hill Book Company, 1977.
[11]. Wenclawiak, B. W.; Giegold, S.; Teutenberg, T. *Anal. Lett.* **2008**, *41*, 1097-1105.
[12]. McNeff, C. V.; Yan, B.; Stoll, D. R.; Henry, R. *J. Sep. Sci.* **2007**, *30*, 1672-1685.
[13]. Yang, Y. *LC GC N. Am.* **2008**, *26-S4*, 2-8.
[14]. Knox, J. H. *J. Chromatogr. Sci.* **1977**, *15*, 352-364.
[15]. Shaaban, H. *Anal. Bioanal. Chem.* **2016**, *408*, 6929-6944.
[16]. Yang, Y. *Anal. Chim. Acta* **2006**, *558*, 7-10.
[17]. He, P.; Yang, Y. *J. Chromatogr. A* **2003,** *989*, 55-63.
[18]. Akay, S.; Odabaşı, M.; Yang, Y.; Kayan, B. *Sep. Purif. Technol.* **2015**, *152*, 1-6.
[19]. Mourilhe, P.; Stokes, P. E. *Drug Saf.* **1998**, *18*, 57-82.
[20]. Cowen, P. J.; Browning, M. *World Psychiatry* **2015**, *14*, 158-160.
[21]. Reilly, J. G.; McTavish, S. F.; Young, A. H. *J. Psychopharmacol.* **1997**, *11*, 381-392.
[22]. Lane, R.; Baldwin, D.; Preskorn, S. *J. Psychopharmacol.* **1995**, *9*, 163-178.
[23]. Gunasekara, N. S.; Noble, S.; Benfield, P. *Drugs.* **1998**, *55*, 85-120.
[24]. Zhang, Y.; Raap, D. K.; Garcia, F.; Serres, F.; Ma, Q.; Battaglia, G.; Van de Kar, L. D. *Brain Res.* **2000**, *855*, 58-66.
[25]. Raap, D. K.; Van de Kar, L. D. *Life Sci.* **1999**, *65*, 1217-1235.
[26]. Eap, C. B.; Baumann, P. *J. Chromatogr. B* **1996**, *686*, 51-63.
[27]. Lacassie, E.; Gaulier, J. M.; Marquet, P.; Rabatel, J. F.; Lachatre, G. *J. Chromatogr. B Biomed. Sci. Appl.* **2000**, *742*, 229-238.
[28]. Tournel, G.; Houdret, N.; Hedouin, V.; Deveau, M.; Gosset, D.; Lhermitte, M. *J. Chromatogr. B Biomed. Sci. Appl.* **2001**, *761*, 147-158.
[29]. Titier, K.; Castaing, N.; Scotto-Gomez, E.; Pehourcq, F.; Moore, N.; Molimard, M. *Ther. Drug. Monit.* **2003**, *25*, 581-587.
[30]. Lucca, A.; Gentilini, G.; Lopez-Silva, S.; Soldarini, A. *Ther. Drug. Monit.* **2000**, *22*, 271-276.
[31]. Petrovic, M.; Skrbic, B.; Zivancev, J.; Ferrando-Climent, L.; Barcelo, D. *Sci. Total Environ.* **2014**, *468-469*, 415-428.
[32]. Eap, C. B.; Bouchoux, G.; Amey, M.; Cochard, N.; Savary, L.; Baumann, P. *J. Chromatogr. Sci.* **1998**, *36*, 365-371.
[33]. Jones, A.; Yang, Y. *Anal. Chim. Acta* **2003**, *485*, 51-55.
[34]. Akay, S.; Kayan, B.; Yang, Y. *J. Chem. Eng. Data* **2017**, *62*, 1538-1543.
[35]. Kapalavavi, B. Fundamental and Applied Studies on Chromatographic Separation of Cold Drugs and Skincare Creams and Extraction of *Salvia Miltiorrhiza* Using Subcritical Water, The Scholarship ECU, 2014.