ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2015, *60*(9), 891-898

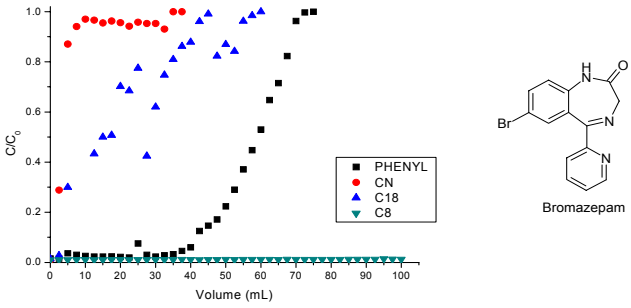
RETENTION BEHAVIOUR OF SOME BENZODIAZEPINES IN SOLID-PHASE EXTRACTION USING MODIFIED SILICA ADSORBENTS HAVING VARIOUS HYDROPHOBICITIES

Elena BACALUM,[a] Mihaela CHEREGI[b,*] and Victor DAVID[b,*]

[a] Research Institute from University of Bucharest – ICUB, 36-46 M. Kogalniceanu Blvd., Bucharest, 050107, Roumania
[b] University of Bucharest, Faculty of Chemistry, Department of Analytical Chemistry, 90 Panduri Ave, Bucharest – 050663, Roumania

Received April 6, 2015

The retention properties of six benzodiazepines (alprazolam, bromazepam, diazepam, flunitrazepam, medazepam, and nitrazepam) on four different solid phase extraction silica adsorbents with various hydrophobicities (octadecylsilica, octylsilica, phenylsilica, and cyanopropylsilica) were investigated. The breakthrough curves showed a significant retention of these compounds on octadecylsilica, octylsilica, phenylsilica, excepting alprazolam that has a poor retention on octadecylsilica. These results can be explained by the hydrophobic character of studied benzodiazepines (octanol-water partition constant, log K_{ow}, being situated within the interval 1.90-4.45). A poor retention on cyanopropylsilica was observed for all studied compounds indicating that π-π and polar intermolecular interactions have a less significant role in their retention on this adsorbent. Generally, the breakthrough curves followed the theoretical shape, with some exceptions due to fluctuations in of the flow-rate of sample loading on the adsorbent bed.



INTRODUCTION

Solid-phase extraction (SPE) is a sample preparation technique widely used for isolation, concentration, clean-up of different samples in various matrices, based on the same principles of liquid chromatography. Currently there is available an increasing number of adsorbents based on low-specificity inorganic oxides (chemically bonded silica) and compound or group-selective materials (such as mixed mode adsorbents, molecularly imprinted polymers, restricted access materials, and immunoaffinity adsorbents), which allow the development of extraction procedures for specific applications.[1]

Nowadays, SPE is the most popular sample preparation technique for both nonpolar and polar analytes due to advantages, such as high selectivity, use of less organic solvents, rare emulsion, minimizes time, cleaner extracts, reproducibility of data, and higher throughput by automatization.[2] SPE can be viewed as a simple

* Corresponding authors: vict_david@yahoo.com; m_cheregi@yahoo.com

chromatographic process with distribution process performed on a very short column with a low number of theoretical plate, but involving compounds with very different distribution coefficients, in which the adsorbent plays the role of the stationary phase and the mobile phase is the solvent of the sample during the adsorption step or a selected solvent during the desorption step.[2-4]

The choice of adsorbent which depends strongly on the analytes of interest is the key point in SPE because it can control parameters such as selectivity, affinity and capacity. However, it also depends on the kind of sample matrix and its interactions with both the adsorbent and the analyte.[3]

Also, the knowledge of retention mechanism and the behavior of different adsorbent are important in solid phase extraction. A specific parameter used to characterize the retention process in SPE is the breakthrough volume established from the breakthrough curve; used to determine the appropriate adsorbents for isolating particular analytes.[5] The breakthrough volume depends on kinetic parameters of adsorbent and on the retention parameters.[6]

The aim of this study was to investigate the retention behaviour of six 1,4-benzodiazepines (namely, alprazolam, bromazepam, diazepam, flunitrazepam, medazepam, and nitrazepam) on four SPE adsorbents with different polarity and hydrophobicity (octadecylsilica, octylsilica, phenylsilica, and cyanopropylsilica). These compounds are prescribed drugs worldwide and have been extensively studied from their detection point of view, such as by mass-spectrometric techniques,[7] or from the chromatographic behaviour in reversed-phase mechanism.[8] This study includes the investigations for adsorption/desorption yields measured on four mentioned adsorbents.

EXPERIMENTAL

Six benzodiazepines (alprazolam, bromazepam, diazepam, flunitrazepam, medazepam, and nitrazepam) were investigated, which were kindly offered by LaborMed Pharma S.A. Their structures are presented in Fig. 1. Water (resistivity minimum 18.2 MΩ and TOC maximum 30 ppb) was produced with a TKA Lab HP 6UV/UF instrument in the laboratory. Acetonitrile and methanol (HPLC grade) was purchased from Sigma Aldrich.

The major molecular parameter indicating the affinity of a compound towards hydrophobic surface, *i.e.* octanol-water partition coefficients (log K_{ow}),[9] for the six studied benzodiazepines are shown in Table 1, together with their pK_a values. Bromazepam and flunitrazepam have similar log K_{ow} values.

Stock standard solutions of 2 mM of benzodiazepines were prepared in methanol. Working solutions of 20 μM were prepared by further dilution in water. Stock standard solutions of 400 ppm benzodiazepines in methanol were prepared that were used to obtain working solutions of 20 ppm by dilution in water.

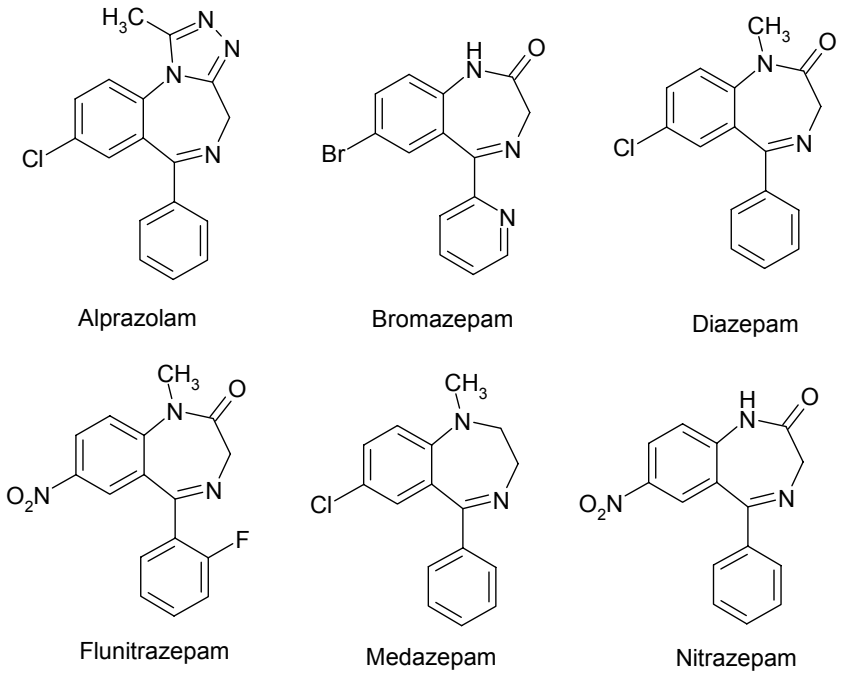


Fig. 1 – Chemical structures of studied benzodiazepines.

Table 1

The values of log K_{ow} for benzodiazepines studied,[10] and pK_a[11-13]

Benzodiazepines	CAS Number	Molecular weight	log K_{ow}	pK_a
Alprazolam	28981-97-7	308.77	3.87	2.4
Bromazepam	1812-30-2	316.16	1.93	2.9
Diazepam	439-14-5	284.74	2.70	3.3
Flunitrazepam	1622-62-4	313.29	1.91	1.8
Medazepam	2898-12-6	270.76	4.43	6.2
Nitrazepam	146-22-5	281.27	2.45	3.2

Table 2

Characteristics of silica based adsorbents used in this study

Cartridge type	Adsorbent mass mg	Surface area m^2/g	Particle size μm	Average pore size Å	Carbon loading %	Retention mechanism	Type of interaction
SampliQ C18	200	541	45	60	25.7	Reversed phase	Hydrophobic
SampliQ C8	200	571	45	60	15.6	Reversed phase	Hydrophobic
SampliQ Phenyl	200	562	45	60	12.6	Reversed phase	Hydrophobic and π-π
Finisterre Cyano	200	n.a*	50	60	4	Reversed phase and normal phase	π-π, dipole-dipole, and hydrophobic

* – not available

An off-line automated solid-phase extraction SPE HT400E system was used. The SPE off-line procedure was described in detail elsewhere.[14] First the adsorbent was conditioned with 5 mL methanol and then accommodating with 5 mL water. The volume of the individual solution of benzodiazepines added was 2.5 mL. Fractions of 2.5 mL were collected until the loss of analyte could be observed, indicating saturation of the adsorbent bed. The adsorbent was never allowed to dry during either the conditioning or sample loading steps. All SPE experiments were performed at room temperature. The flow rate was kept constant during the experiments at 1 mL/min.

Four types of adsorbents containing octadecyl (C18), octyl (C8), phenyl and cyanopropyl silica have been used; with the physico-chemical properties presented in Table 2. Chemically bonded silicas, usually with C8 and C18 organic groups, are by far the most commonly used SPE materials. Octadecylsilica adsorbent is considered one of the most non-selective adsorbents.

The absorption spectra of benzodiazepines were recorded with a Jasco V-530 double beam spectrometer, in 1 cm quartz cells, and used to choice of the maximum sensitivity of UV detection of these benzodiazepines. The absorbance were measured at λ_{max} = 220 nm for alprazolam, at λ_{max} = 226 nm for bromazepam and diazepam, and at λ_{max} = 254 nm for flunitrazepam, medazepam, and nitrazepam.

RESULTS AND DISCUSSION

Many SPE procedures for extraction of benzodiazepines in various sample matrixes are reported in the literature.[15,16] These procedures include uses of C2, C8, C18,[17-20] mixed-mode columns[21] and polar cyano adsorbents.[22] Beside usually used silica based adsorbents; polymeric cartridges, hydrophilic modified reversed-phase (Oasis HLB, a hydrophilic–lipophilic balanced copolymer)[23] cartridge and adsorbents containing a cationic-exchange group or anionic-exchange functionalities were investigated for the solid-phase extraction of some benzodiazepines in serum and urine.[24,25] Recently, molecularly imprinted solid-phase extraction (MISPE),[26,27] on-line enrichment and clean-up on a restricted access extraction column,[28,29] and multiwalled carbon nanotubes (MWCNTs) adsorbents[30] for benzodiazepines extraction were also used. Parallel extraction of benzodiazepines was also accomplished on a 96-well disk solid phase extraction technique.[31] Although benzodiazepines have structural similarities, they have various behaviors towards adsorbents used in SPE because of differences in polarity.[32]

This paper is focused on the investigation of breakthrough curve of six benzodiazepines on four different types of SPE silica adsorbents (octadecyl, octyl-, phenyl- and cyanopropylsilica). As presented in Table 2, the four used adsorbents are involved in the retention mechanisms including: hydrophobic, π-π and dipole-dipole. Among them, π-π interactions may have a great contribution to the retention on both cyano and phenyl adsorbents. Dipole-dipole interactions can be dominant for cyano columns (due to increased dipole moment of the cyano group).

The breakthrough curve is obtained by percolating the SPE cartridge containing a specified adsorbent with a test solution containing the analyte at concentration level denoted by C_0. The analyte concentration in eluate is measured by means of spectrometric method. The equation describing the breakthrough curve is a Boltzmann function, written in the form:[3]

$$C \quad C_0 \quad \frac{A \quad C_0}{1 \quad \exp(\frac{V \quad V_R}{B})} \qquad (1)$$

In this equation, V_R is the retention volume, A and B are empirical parameters obtained from the best fit of the experimental curve with Boltzmann function.[33] According to this equation, in the point $V = V_R$, the value of C becomes $C = (C_0 + A)/2$. However, many times in practice the curve does not obey to such a dependence, which might be assigned to the complex process of the analyte adsorption/desorption on the adsorbent.[6,34]

In this work, the breakthrough curves were studied for the six benzodiazepines. The breakthrough volume (V_b), the maximum volume samples that can be preconcentrated on a adsorbent without producing losses of any particular compound, is different for each analyte is the most important factor in determining the suitability of a adsorbent for SPE and it depends on the analyte hydrophobicity and the mass of the adsorbent used. The breakthrough volume V_b is defined as the volume of sample that has passed through a adsorbent bed until 1% of the analyte concentration is measured at the outlet.[4,5] The breakthrough curves obtained for alprazolam obtained on the four adsorbents described in Experimental are given in Fig. 2.

Alprazolam, a triazolo benzodiazepine, is retained strongly on octyl and phenylsilica-based cartridges, possible due to the favorable hydrophobic interaction and π-π interactions; while the retention on the CN cartridge was very poor, and the retention on C18 adsorbents was low. Although octylsilica has a lower carbon loading compared with octadecylsilica, C8 has a greater surface area that C18 which could explain the stronger retention of alprazolam on octylsilica.[35] Retention on octylsilica is one very favorable. The carbon loading of cyanosilica cartridges is small (see Table 1) and the retention of alprazolam on this adsorbent is minimum. Therefore, alprazolam can not be adsorbed on the surface of polar adsorbent such as cyanopropylsilica.

Bromazepam is less hydrophobic than alprazolam, according to octanol-water partition coefficient from Table 1, having one deprotonation site (an amide group) and two possible protonation sites (imines and pyridine nitrogen). It may be expected to observe a weaker adsorption of bromazepam on C8 and C18 silica based adsorbents and a stronger adsorption on cyanopropylsilica adsorbent compared to alprazolam. The breakthrough curves for bromazepam obtained in the same conditions as for alprazolam are shown in Fig. 3. A similar retention behavior with alprazolam was observerd for bromazepam on C8 versus C18 silica adsorbents, with the remark that the breakthrough curve obtained for bromazepam on C18 was more irregular than in case of alprazolam. However, a sigmoidal shaped breakthrough curve closer to theoretical shape has been obtained for bromazepam on phenyl cartridge. The strongest retention (retention volume more than 100 mL) of bromazepam was observed on octylsilica, due to favorable hydrophobic interaction with the octyl chain from the adsorbent surface. The phenyl moiety allows strong interaction between bromazepam and phenyl silica adsorbent due to the π-π stacking interactions, which are however of low intensity and consequently the breakthrough of the cartridge takes place after a small volume of sample (about 40 mL). The breakthrough curve obtained on cyanosilica may be a proof that in this case the polar interactions between bromazepam and cyanopropyl moiety are practically negligible.

Diazepam, a benzodiazepine characterized by the presence of a ketone group in position 2, a methyl moiety at nitrogen in position 1, and a chlorine atom at position 8, has a moderate hydrophobicity. The breakthrough curves obtained for diazepam on the four adsorbents are depicted in Fig 4. The retention of diazepam on C_6H_5-, C8, and C18 silica adsorbents were high, so that the breakthrough did not take place even after 80 mL of loaded diazepam solution.

Similar to alprazolam and bromazepam, retention of diazepam on cyanosilica based adsorbent was poor. Although diazepam has some polar moieties its interaction with the polar cyanopropyl moiety on the adsorbent surface seems not possible or being in competition with polar interactions between diazepam and molecule of the sample solvent. Diazepam can interact with the phenyl rings from the adsorbent surface by π-π stacking interactions.

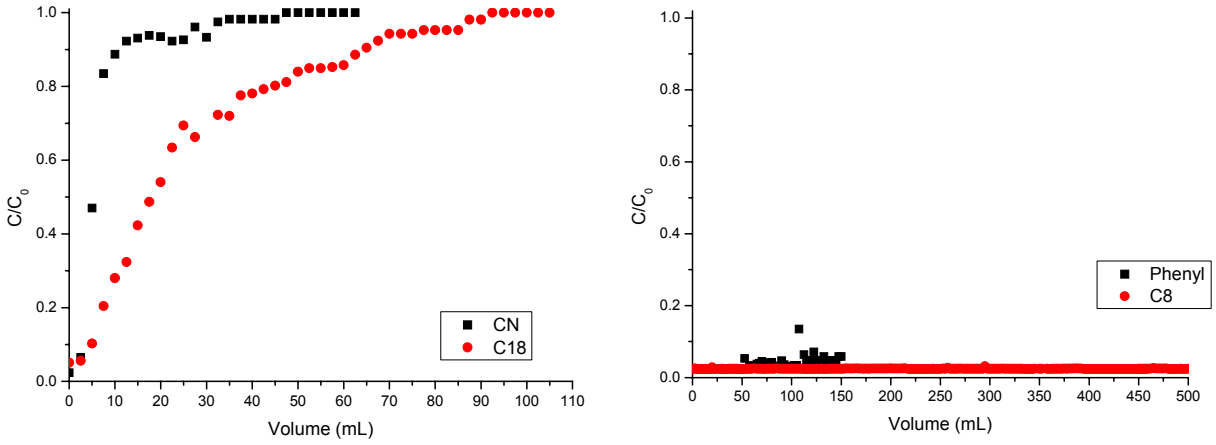


Fig. 2 – The breakthrough curves obtained for alprazolam on four silica adsorbents.

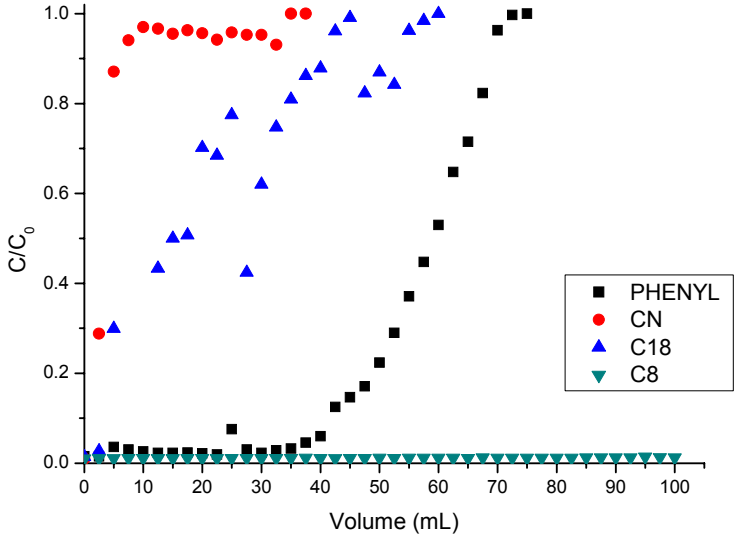


Fig. 3 – The breakthrough curves obtained for bromazepam on four silica adsorbents.

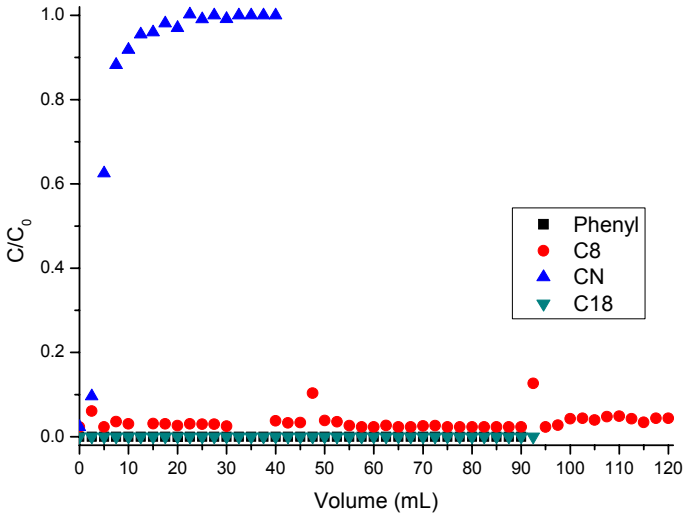


Fig. 4 – The breakthrough curves obtained for diazepam on four silica adsorbents.

The elution curves for flunitrazepam on the four adsorbents used in this study are presented in Fig 5. Similar to diazepam, flunitrazepam is a keto derivatives characterized by the presence of a ketone moiety in position 2. Flunitrazepam is also characterized by the presence of the methyl moiety attached to nitrogen in position 1 and a fluorine atom is found at position 5. Flunitrazepam has the lowest hydrophobicity from the benzodiazepines studied (log K_{ow} = 1.91), but it is sufficient highly to ensure the hydrophobic interactions with C8 and C18 adsorbents. Similar to the previous discussed benzodiazepines, flunitrazepam can interact strongly with phenyl rings from the adsorbent surface by means of π-π interactions, and poorly retained on cyanopropyl adsorbent.

Medazepam is a deoxy derivative of diazepam and has the highest hydrophobicity among the studied benzodiazepines. The elution curves for medazepam on the four adsorbents used in this study are pointed out in Fig 6. Its retention behavior is similar to the other studied compounds, excepting a slight interaction of medazepam with CN adsorbent that can be observed from breakthrough curve for cartridge.

Nitrazepam is keto derivatives characterized by the presence of a ketone in position 2. Nitrazepam contains an additional nitro moiety at position 8. The presence of a nitro moiety at position 7 for nitrazepam can create an acidic character at nitrogen in position 1. The breakthrough curves obtained for nitrazepam on the four adsorbents are depicted in Fig 7. Again, the lowest retention was obtained on cyanopropyl silica and high retentions were obtained for phenyl, C8 and C18 silica based adsorbents which can indicate a strong interactions with these adsorbents.

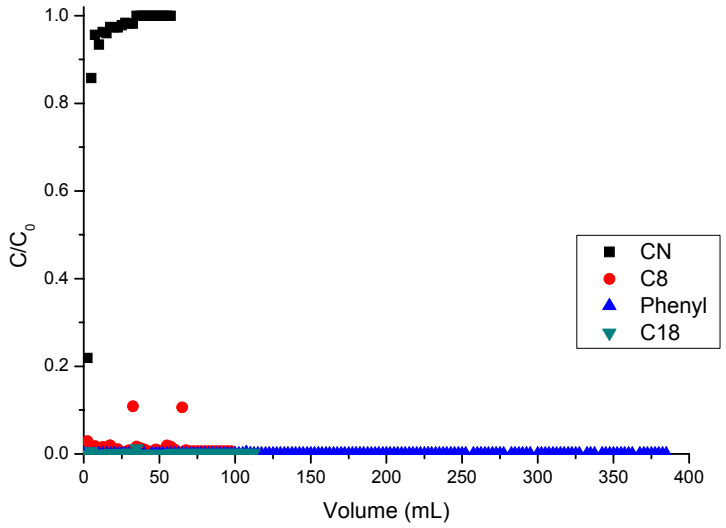


Fig. 5 – The breakthrough curves obtained for flunitrazepam on four silica adsorbent.

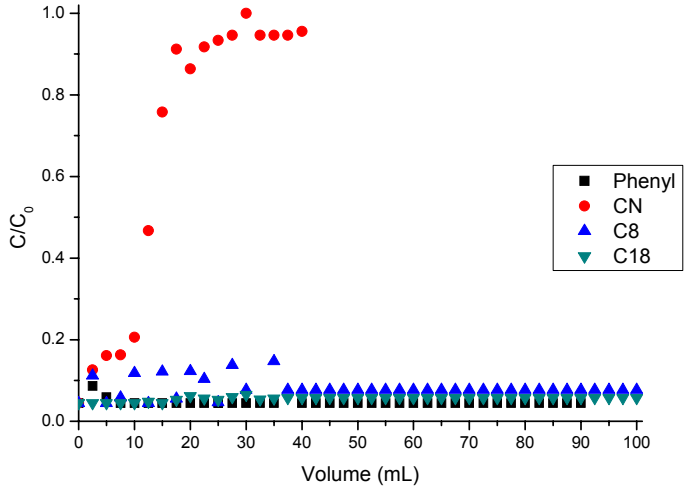


Fig. 6 – The breakthrough curves obtained for medazepam on four silica adsorbents.

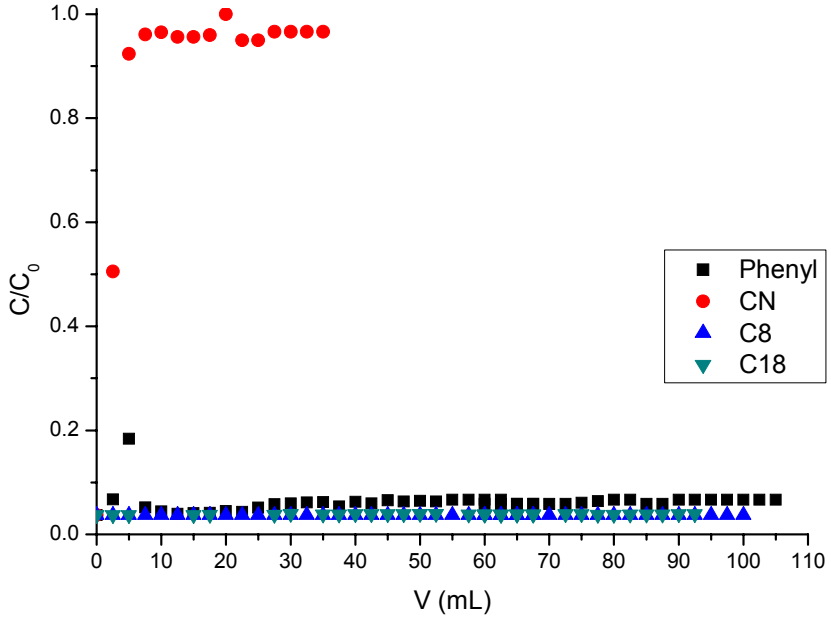


Fig. 7 – The breakthrough curves obtained for nitrazepam on four silica adsorbents.

From the comparison of the four tested SPE cartridges it was concluded that the highest averaged analytical recovery (defined as the ratio between the amount extracted and the amount applied) was achieved with octylsilica sorbent in agreement with breakthrough curves presented in Figs. 2-7. Adsorption yield varied from 51% obtained for nitrazepam on CN adsorbent and 100% for all studied benzodiazepines on octyl or phenylsilica adsorbents. The desorption yields using methanol as extraction solvent varied between 78% for bromazepam on cyanosilica adsorbent and 100% for almost all benzodiazepines on octyl or phenylsilica adsorbents. Generally, the parameters that influence both adsorption and desorption of analytes in SPE are the hydrophobicity of adsorbent and solvent, Hansen solubility parameters of analyte and the breakthrough volume values.[36,37]

CONCLUSIONS

The retention of six 1,4-benzodiazepines on four silica based adsorbents indicates that the benzodiazepines are strongly retained on three (phenylsilica, octylsilica, octadecylsilica) of the four adsorbent, with large retention volume achieved. The retention of benzodiazepines on the adsorbents is primary governed by hydrophobic interactions, and π-π stacking in case of phenylsilica adsorbent. The sigmoid-like shape expected for SPE breakthrough curves was obtained only for some cases. Generally, the breakthrough curves followed the theoretical shape, with some exceptions due to fluctuations in the flow-rate of sample loading on the adsorbent bed that may influence the adsorption/desorption equilibrium of studied compound on the surface of adsorbent. Such deviations from the theoretical shape has been reported by several studies in literature for other compounds and adsorbents, which in several experiments the points were situated very far from the theoretical breakthrough curve.[34]

Acknowledgements: The authors acknowledge the financial support to a grant provided by the Roumanian National Authority for Scientific Research, CNCS – UEFISCDI, project PN-II-PT-PCCA, no. 197/2014. One of the authors (Dr. E. Bacalum) acknowledges the financial support received from the Research Institute from University of Bucharest (ICUB) within the framework of a project for young researchers (2015).

REFERENCES

1. C. F. Poole, *TrAC - Trends in Anal. Chem*, **2003**, *22*, 362-373.
2. M. C. Hennion, *J. Chromatogr. A*, **1999**, *856*, 3-54.
3. S. Moldoveanu and V. David, "Solid-Phase Extraction", in "Modern Sample Preparation in Chromatography", Elsevier, Amsterdam, 2015, p. 191-286.
4. B. Buszewski and M. Szultka, *Crit. Rev. Anal. Chem.*, **2012**, *42*, 198-213.
5. C. F. Poole, A.D. Gunatilleka and R. Sethuraman, *J. Chromatogr. A*, **2000**, *885*, 17-39.
6. K. Bielicka-Daszkiewicz and A. Voelkel, *Talanta*, **2009**, *80*, 614-621.

7. T. Galaon, C. Vacaresteanu, D. F. Anghel and V. David, *Drug Testing and Analysis*, **2014**, *6*, 439-450.
8. T. Galaon, D. F. Anghel, V. David and H. Y. Aboul-Enein, *Chromatographia*, **2013**, *76*, 1623-1630.
9. V. David and A. Medvedovici, *J. Liq. Chromatogr. Rel. Technol*., **2007**, *30*, 761-789.
10. http://www.ttsys.com/episuite.html (EPI Suite Software)
11. P. Campíns-Falcó, A. Sevillano-Cabeza, R. Herráez-Hernández, C. Molins-Legua, Y. Moliner-Martínez and J. Verdú-Andrés, "Solid-Phase Extraction and Clean-Up Procedures in Pharmaceutical Analysis", in "Encyclopedia of Analytical Chemistry", John Wiley & Sons, New-York, 2012, p. 1-10.
12. A. Catabay, M. Taniguchi, K. Jinno, J. J. Pesek and E. Williamsen, *J. Chromatogr. Sci.*, **1998**, *36*, 111-118.
13. U. Chiuminatto, F. Gosetti, P. Dossetto, E. Mazzucco, D. Zampieri, E. Robotti, M. C. Gennaro and E. Marengo, *Anal. Chem.,* **2010**, *82*, 5636-5645.
14. E. Bacalum, M. Radulescu, E. E. Iorgulescu and V. David, *Rev. Roum. Chim.*, **2011**, *56*, 137-143.
15. M. Nakamura, *Biomed. Chromatogr.*, **2011**, *25*, 1283-1307.
16. L. A. Berrueta, B. Gallo and F. Vicente, *J. Pharm. Biomed. Anal*, **1992**, *10*, 109-136.
17. M. J. Bogusz (Ed.), "Forensic Science, Handbook of Analytical Separations", Vol. 6, Elsevier, 2008, p. 252.
18. K. B. Borges, E. F. Freire, I. Martins and M. E. P. Bastos de Siqueira, *Talanta*, **2009**, *78,* 233-241.
19. M. N. Uddin, V. F. Samanidou and I. N. Papadoyannis, *J. Sep. Sci.*, **2008**, *31*, 3704-3717.
20. M. Casas, L. A. Berrueta, B. Gallo and F. Vicente, *J. Pharm. Biomed. Anal.,* **1993**, *11*, 277-284.
21. N. Karlonas, A. Padarauskas, A. Ramanavicius and A. Ramanaviciene, *J. Sep. Sci.*, **2013**, *36*, 1437-1445.
22. O. H. Drummer, *J. Chromatogr. B*, **1998**, *713*, 201-225.
23. H. Inoue, Y. Maeno, M. Iwasa, R. Matoba and M. Nagao, *Forensic Sci. Int.*, **2000**, *113*, 367-373.
24. H. He, C. Sun, X-R. Wang, C. Pham-Huy, N. Chikhi-Chorfi, H. Galons, M. Thevenin, J.R. Claude and J. M. Warnet, *J. Chromatogr. B*, **2005**, *814*, 385-391.
25. I. Racamonde, R. Rodil, J. B. Quintana, E. Villaverde-de-Saa and R. Cela, *J. Chromatogr. A*, **2014**, *1352*, 69-79.
26. M. M. Ariffin, E. I. Miller, P. A. G. Cormack and R. A. Anderson, *Anal. Chem.*, **2007**, *79*, 256-262.
27. R. A. Anderson, M. M. Ariffin, P. A. G. Cormack and E. I. Miller, *Forensic Sci. Int.*, **2008**, *174*, 40-46.
28. W. M. Mullett and J. Pawliszyn, *J. Pharm. Biomed. Anal*., **2001**, *26*, 899-908.
29. A. El Mahjoub and C. Staub, *Forensic Sci. Int.*, **2001**, *123*, 17-25.
30. L. Wang, H. Zhao, Y. Qui and Z. Zhou*, J. Chromatogr. A*, **2006**, *1136*, 99-105.
31. E. Cudjoe and J. Pawliszyn, *J. Pharm. Biomed. Anal.*, **2009**, *50*, 556-562.
32. M. Nakamura, T. Ohmori, Y. Itoh, M. Terashita and K. Hirano, *Biomed. Cromatogr.*, **2009**, *23*, 357-364.
33. E. Bacalum, E. E. Iorgulescu and V. David, *Rev. Roum. Chem.,* **2012**, *57*, 715-720.
34. K. Bielicka-Daszkiewicz, A. Voelkel, D. Rusińska-Roszak and P. K. Zarzycki, *J. Sep. Sci*., **2013**, *36*, 1104-1111.
35. S. C. Moldoveanu and V. David, *J. Sep. Sci*., **2013**, *36*, 2963-2978.
36. K. Bielicka-Daszkiewicz, *J. Sep. Sci*., **2015**, *38*, 439-444.
37. K. Bielicka-Daszkiewicz, A. Voelkel, M. Pietrzyńska and K. Heberger, *J. Chromatogr. A*, **2010**, *1217*, 5564-5570.