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UNIVERSITY OF BUCHAREST
FACULTY OF CHEMISTRY
CHEMISTRY DOCTORAL SCHOOOL

DOCTORAL THESIS

COMPARATIVE RETENTION STUDIES OF POLAR COMPOUNDS BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY

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2012

SYNOPSIS

THESIS SYNOPSIS

The doctoral thesis has been divided into two main chapter the first one present some theoretical and experimental aspects of the solid phase extraction techniques and give a short presentation on the retention mechanism in liquid chromatography and the second one is the experimental part.

The first chapter of the experimental part present the retention studies of four polar compounds (atenolol, metoprolol, pentoxifylline, and captopril) on two octadecyl silica based sorbents (Ultra Clean C18 – 200 m and Bond Elut C18 – 2g) with different mass. From the breakthrough curves the SPE parameters (breakthrough volume, retention volume and final volume) have been calculated for each compounds. The breakthrough curves were modeled by Boltzmann's function, and the main regression parameters were used in calculating the retention parameters. For atenolol on the Bond Elut C18 cartridges the breakthrough curve profile has an abnormal shape with maximum and minimum which can be explained by the multiple adsorption-desorption equilibrium.

For metoprolol and pentoxifylline similar theoretical sigmoidal breakthrough curves have been observed. For captopril the breakthrough volume on Ultra Clean C18 cartridge was much smaller then on a Bond Elut C18 cartridge which can be explained by the captopril oxidation to disulphide form. In *Figure 18* is presented the breakthrough curves obtained for pentoxifylline on a Ultra Clean C18.

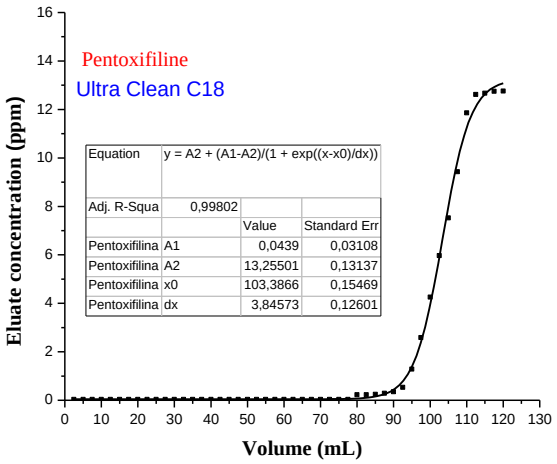

Figure 18. Dependence of pentoxifylline concentration 2.5 mL volume loading of aqueous samples and modeling by Boltzmann function

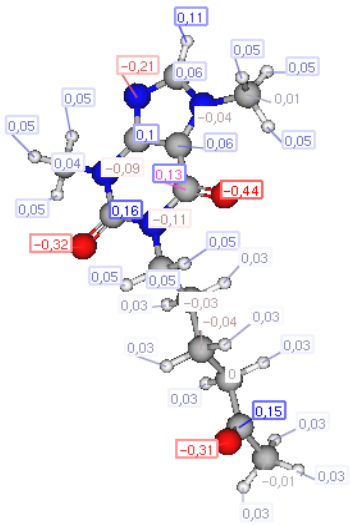

Figure 19. Pentoxifylline structure and atoms charged obtained by Marvin program (carbon - grey, hydrogen - white, nitrogen - blue, oxygen - red)

The highest values of breakthrough volume was obtained for the Bond Elut C18 sorbents in the case off all compounds. The molecular modeling with the aid of Marvin program shown the charges of atoms which can be used to give an insight into explaining the retention mechanism. The compounds are retained on the octadecyl silica based cartridges due to hydrophobic interactions and hydrogen bonding with the silanol groups of sorbents.

The study of the influence of pH on retention properties in the solid-phase extraction of basic compounds indicate that although the breakthrough volume for the neutral and acid solution has a similar shape in the case on metoprolol the breakthrough volume in acid media is much smaller that in neutral solution.

Retention of polar compounds on octadecyl silica based sorbents can be enhanced by adding an ion pairing reagents (hexan and heptan sulphonate) similar with the procedure used in liquid chromatography. The retention volume obtained for the metformin indicate that the ion pair reagents does not significantly influence the retention properties of studied compound.

Analytical procedures for enrichment of atenolol, metoprolol, and pentoxifylline from aqueous solutions were performed at different concentration levels. The slope of calibration function for metoprolol was lower then that obtained for atenolol, although the compounds have similar behavior in the adsorption process.

In the second chapter of the experimental part retention of five polar compounds (ciprofloxacin, norfloxacin, metformin, o-tolyl biguanide and gliquidone) have been investigated on four silica based sorbents (octadecyl-silica, octyl-silica, phenyl-silica and cyanopropyl-silica). Comparative retention curves of compounds on chosen sorbents showed different behavior, which are not always correlated with structure. The retention parameters have been calculated for the studied compounds. Ciprofloxacin and norfloxacin are retained on the C8 and C18 sorbents because hydrophobic interaction with the sorbents and the silanol groups of silica sorbents. The study indicate that the polarities of the compounds has an influence on the retention profile. The retention mechanism of compounds on octadecyl-silica and octyl-silica is due to hydrophobic interaction, on the cyanopropyl silica the retention mechanism is due to dipol-dipol interactions. On phenyl silica the retention of compounds is due to $\pi-\pi$ interaction beside hydrophobic interaction.

For ciprofloxacin and norfloxacin similar profile of breakthrough curves four all sorbents have been obtained. The retention on octadecylsilica and octyl based sorbents are due to hydrophobic interactions and on $\pi-\pi$ interaction on phenyl type cartridges. In the case of ciprofloxacin and norfloxacin the strong interaction with the octyl-silica sorbents could be explained by lower hydrophobic character of C8 compared to C18 sorbents. For metformin and o-tolyl biguanide the breakthrough curves obtained has a sigmoidal theoretical profile. In the case of

o-tolyl biguanide on the cyanopropyl silica cartridges was obtained a two step profile. The charges of ciprofloxacin do not allow van der Waals interactions with octadecyl chain (see *Figure 40*).

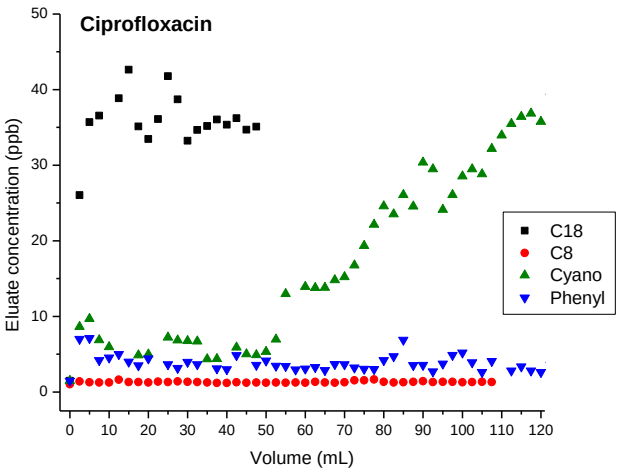

Figure 39. Dependence of ciprofloxacin concentration on succesive 2.5 mL volume loading of aqueous samples

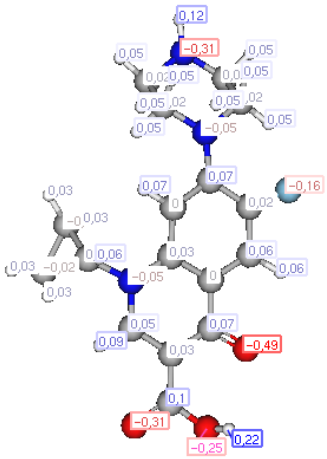

Figure 40. Ciprofloxacin structure with atoms charges obtained by Marvin program (carbon - grey, hydrogen - white, nitrogen - blue, oxygen - red)

Metformin although is a compound that has a hydrophobic moiety (i.e. phenyl ring) it exhibited no affinity towards C18 sorbent. Tolyl biguanide can interact with the phenyl type silica cartridges, octyl-silica and octadecyl-silica sorbents by means of hydrophobic and π–π interactions.

Gliquidone which is the most hydrophobic compounds from the study has a strong interaction with the C18, C8 and phenyl type cartridges and a low interaction with the cyanopropyl cartridges. Although gliquidone has some negative charges on its molecule its interaction with polar groups from CN adsorbent surface seems not possible to occur because of steric hindrance.

Molecular modeling of the studied molecules with the aid of Marvin program revealed the charged centers (oxygen atoms, fluor atoms) on these molecules, which can be involved in dipole-dipole interactions with sorbent (cyano group, or residual silanol from silica surface).

In the third chapter a comparative retention studies of six benzodiazepine (alprazolam, bromazepam, diazepam, flunitrazepam, medazepam and nitrazepam) on four silica sorbents was performed by solid phase extraction and liquid chromatography.

The solid phase extraction study performed indicate that the retention of benzodiazepine on octadecyl-silica, octyl-silica and phenyl-silica was due to hydrophobic interaction and π–π

interactions. The retention of benzodiazepines on cyanopropyl silica was small possible because of the bulkiness of the molecule and steric hindrance. Although the studied compounds have different hydrophobicity they have similar behavoir on the sorbents used in the study.

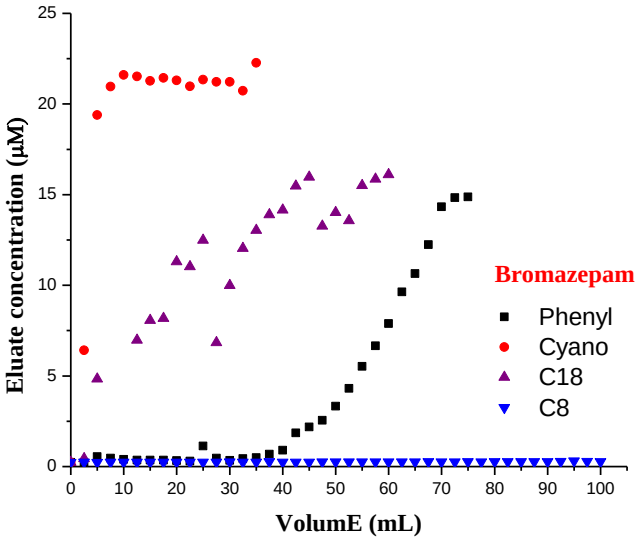

Figure 54. Dependence of bromazepam concentration on succesive 2.5 mL volume loading of aqueous solution

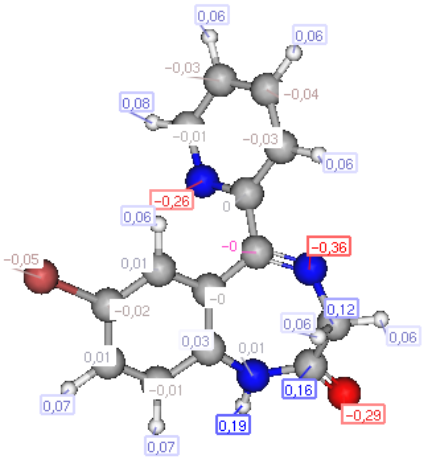

Figure 55. Bromazepam structure with atoms charges obtained by Marvin program (carbon - grey, hydrogen - white, nitrogen - blue, oxygen - red, bromine – dark red)

The liquid chromatographic retention studies of benzodiazepines on four silica based stationary phases (similar with the ones used in solid-phase extraction studies) with acetonitril and methanol as organic modifier of mobile phase allowed the calculation of retention factor at a 100 % aqueous mobile phases. This parameters can be correlated with the retention volume from the solid-phase extraction procedure, see table 37.

Table 37. Comparison of retention of six benzodiazepines obtained by HPLC and SPE techniques

Compound	**HPLC Retention (log k_w)**				**SPE Retention (V_B - mL)**			
	C18	**C8**	**C_6H_5**	**CN**	**C18**	**C8**	**C_6H_5**	**CN**
Alprazolam	4.31	4.27	3.38	2.51	5	> 670	> 120	1
Bromazepam	3.55	3.40	1.96	0.70	5	> 100	28	1
Diazepam	4.81	4.77	3.76	2.54	> 35	> 35	> 60	1
Flunitrazepam	3.99	3.99	4.53	2.28	> 60	> 375	> 75	1
Medazepam	5.51	5.62	5.44	1.39	> 35	> 35	> 35	5
Nitrazepam	3.72	3.59	2.40	2.13	> 80	> 55	> 55	1

In the fourth chapter a retention studies of thirteen compounds with or without halogens atoms in the molecule (2-chlorphenol, 2,4-chlorphenol, 2,6-chlorphenol, 1,4-dichlorbenzene, 1,2,4,5-tetrachlorbenzene, simvastatin, lovastatin, indapamid, adenine and four isomers of hexachlorocyclohexane) on a pentafluorophenyl silica stationary phases was performed with the aim to investigate the retention mechanism. For adenine it have been obtained a profile of retention with a sigmoidal profile which is not been reported in the literature. The shape can be explained by amino-imino tautomerism.

The retention study of simvastin, lovastatin and indapamid on pentafluorophenyl silica based sorbents have been investigated on three type of organic modifiers of mobile phases: acetonitril, methanol and acetonitril-methanol. For all the mobile phases composition have been obtained polynomial dependence.

The retention of five halogenated compounds (2-chlorphenol, 2,4-dichlorphenol, 2,6-dichlorphenol, 1,4-dichlorbenzen, 1,2,4,5-tetrachlorbenzen) on the pentafluorophenyl-silica for two different organic modifier of the mobile phases (acetonitril and methanol) have been investigated Polynomial dependence on the retention factor on the organic mobile phases composition have been obtained.

A comparative retention study of the isomers of hexachlorocyclohexane on three stationary phases (C8, C18, and PFP) revealed that the best resolution and separation was obtained for the octadecyl silica based sorbents.

In the fifth part the main purpose was to investigate the effects of large volume of aromatics solvents (benzene, toluene, ethylbenzene, and propylbenzene) on a phenyl type stationary phase. Until now it have been investigate this process only on the octadecyl-silica sorbents. It have been investigated seven aromatics compounds (phenol, acetylsalicylic acid, benzoic acid, benzyl alcohol, codeine, trimetazidine and 4,4-dipiridine). The aromatic solvents are adsorbed on the beginnning of the stationary phases due to $\pi-\pi$ interaction that blocks the adsorption centers which determine a decrease in the retention time of compounds. The study revealed that is possible to inject large volume of aromatics solvents without negative influence on the chromatographic properties (efficiency and symmetry).

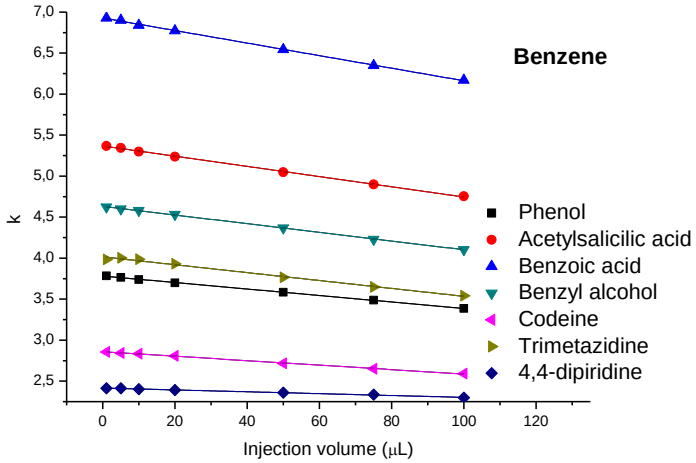

Figure 107. Dependence of retention factor on injection volume for seven compounds with benzene as solvent

Conclusions

The present doctoral thesis investigate the retention properties of organic polar compounds by solid-phase extraction and liquid chromatography. The compounds structure modelated by Marvin program indicate that polarities of the compounds plays an important role in the adsorption process both in solid-phase extraction and in liquid chromatography.

A comparative retention studied performed on benzodiazepines by liquid chromatography on stationary phases similar with that one in the solid-phase extraction shown a strong interaction with the most hydrophobic part of the sorbents.

The role of the polar interaction between the analyte and the stationary phases have been investigated on an pentaflurophenyl-silica cartridges for nine compounds with or without halogen in the molecule. The study shown that the compounds are separated according to their hidrophobicity.

List of articles published or accepted in the doctoral thesis topics

1. E.Bacalum, M.Rădulescu, E-E.Iorgulescu, V.David, Breakthrough parameters of SPE procedure on C18 cartridges for some polar compounds, *Revue Roumaine de Chimie*, **2011**, *56*, 137-143. (Factor de Impact: 0,418).
2. E.Bacalum, M.Cheregi, V.David, Retention studies of polar compounds in solid-phase extraction on different bonded silica-based adsorbents, *Revue Roumaine de Chimie*, acceptat spre publicare în numărul 57 (4-5), **2012**. (Factor de Impact: 0,418).
3. E.Bacalum, E-E.Iorgulescu, V.David, Enrichment of several benzodiazepines by solid phase extraction with octyl and phenyl silica based adsorbents, *Revue Roumaine de Chimie*, acceptat spre publicare în numărul 57 (7-8), **2012**. (Factor de Impact: 0,418).
4. T.Galaon, E.Bacalum, M.Cheregi, V.David, Retention studies for large volume injection of aromatic solvents on phenyl-silica based stationary phase in RP-LC, *Journal of Chromatographic Science*, acceptat spre publicare, **2012**.
   (Doi: 10.1093/chromsci/BMS122). (Factor de Impact: 0,884).
5. E.Bacalum, A.Tănase, V.David, Retention mechanism applied in solid phase extraction for some polar compounds, *Analele Universităţii din Bucureşti, Seria Chimie*, **2010**, *19*, 61-68.

Scientific Communication

1. Elena Bacalum, Victor David, Comparative retention of some polar compounds on various modified silica based sorbents, Sesiunea de Comunicări Ştiinţifice Studenţeşti, VII edition, 20 may 2011.

2. Elena Bacalum, Victor David, Retention modelling in solid-phase extraction on different types of silica based sorbents, Summer School on Mathematics and Natural Sciences, 11-15 july 2011, Bucharest

3. Elena Bacalum, Toma Galaon, Victor David, Retention studies of some organic compounds on pentafluorophenyl silica based stationary phase, Sesiunea de Comunicări Ştiinţifice Studenţeşti, VIII edition,18 may 2012.