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Dedicated to Professor Ionel Haiduc
on the occasion of his 75th anniversary

RETENTION STUDIES OF POLAR COMPOUNDS IN SOLID-PHASE EXTRACTION ON DIFFERENT BONDED SILICA-BASED ADSORBENTS

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The behavior of five polar compounds was studied by solid-phase extraction on adsorbents of different polarities. The adsorbents employed in this study were based on silica bonded with chains of different hydrophobicity and polarity (octadecyl, octyl, cyanopropyl and phenyl). Comparative retention curves of the model compounds on chosen adsorbents showed different behavior, which are not always correlated with structure. The breakthrough parameters (the hold-up, breakthrough, and retention volumes) were measured for chosen model solutes from the experimental data obtained by percolating the cartridges with stock solutions for each compound at ppm or ppb levels. Structure modeling of the studied compounds was performed with the aid of Marvin program, and allowed the identification of the charged centers on molecular structure. Thus, dimethylguanidine and o-tolylguanidine, which are highly polar compounds, are interacting strongly with cyanopropyl silica adsorbent, while gliquidone, which is a highly hydrophobic compound, is interacting strongly with octadecyl, octyl, and phenyl silica adsorbents. Two studied fluoroquinolones were observed that they are interacting with phenyl silica adsorbent due to the π-π stacking interactions.

INTRODUCTION

Solid phase extraction (SPE) is a method largely used for isolation and concentration of analytes by their transfer to a solid-phase, which confer certain advantages over the liquid-liquid extraction.[1-3] SPE has become a widely used method in the field of drug analysis from a large variety of samples with more or less complex matrix composition.[4-7] Drugs have a wide range of polarity and it should be noted that hydrophobic interactions are not the only retention mechanism possible with reversed phase based cartridges.[8] Other types of interactions that can be involved in the retention mechanism are hydrogen bonding type with residual silanols from bonded silica and π-π interactions with cyano or phenyl bonded groups.[9] In practice, the selection of proper sorbent depends on the nature of target compounds and sample, as well as on a series of other factors that include the selectivity and sensitivity required for the analysis, cost, etc.[10]

The physico-chemical properties of the sorbent determine extraction efficiency and the overall quality of the SPE separation.[11] Differences in the chemical structure and polarity amongst the sorbents can lead to major changes in selectivity, retention and recovery for a given analyte. The information about the hydrophobicity or polarity of compounds can be used to choose the appropriate retention mechanism and then to predict the retention behavior on particular sorbent. The mechanism of surface adsorption is governed by the character of interactions between solutes and active sites of the surface.[12]

The aim of this work was to study the retention behavior of five compounds of different

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hydrophobicity and polarity on different silica based sorbents and to correlate the outcome with compound structure. The influence of polar moieties on the solid phase extraction behavior of chosen compounds has also been studied. These compounds are very important from pharmaceutical point of view, and their determination from complex samples could rely on their isolation and concentration on SPE adsorbents having characteristics in accordance to structural features of these compounds.

EXPERIMENTAL

Materials and methods: Five compounds were studied in this work: ciprofloxacin and norfloxacin (fluoroquinolones antibiotics), metformin and o-tolyl biguanide (biguanides are used for diabetes mellitus treatment) and gliquidone (antidiabetic drug from sulfonylurea class), which were kindly offered by LaborMed Pharma S.A. Their structures are presented in Fig. 1. Methanol and acetonitril, HPLC grade were purchased from Sigma Aldrich. Water (resistivity minimum 18.2 MΩ and TOC maximum 30 ppb) was produced within the laboratory with a TKA Lab HP 6 UV/UF instrument.

Some information concerning the log K_{ow} values and acidity constants are given in Table 1. It can be observed that gliquidone is the most hydrophobic compounds, while metformin is the most hydrophilic.

Preparation of solutions: Stock solutions of 200 ppm were prepared in water for metformin and o-tolyl biguanide, in methanol for gliquidone and in 1% acid acetic aqueous solutions for ciprofloxacin and norfloxacin. Standard solutions of 20 ppm (metformin and o-tolylbiguanide) and 50 ppb (ciprofloxacin, norfloxacin and gliquidone) were prepared by dilution. In order to establish the standard calibration curves, solutions containing individual compounds have been prepared at six levels of concentration.

SPE procedure: The solid phase extraction procedure used for this study was described in a previous work.[14] The sample was introduced in aliquots of 2.5 mL aqueous solutions containing different concentrations of studied compounds (as mentioned in next section). The absorbance (for biguanidines, and gluiqidone) or emission intensity (for fluoroquinolones) of the eluate were measured after each sample loading.

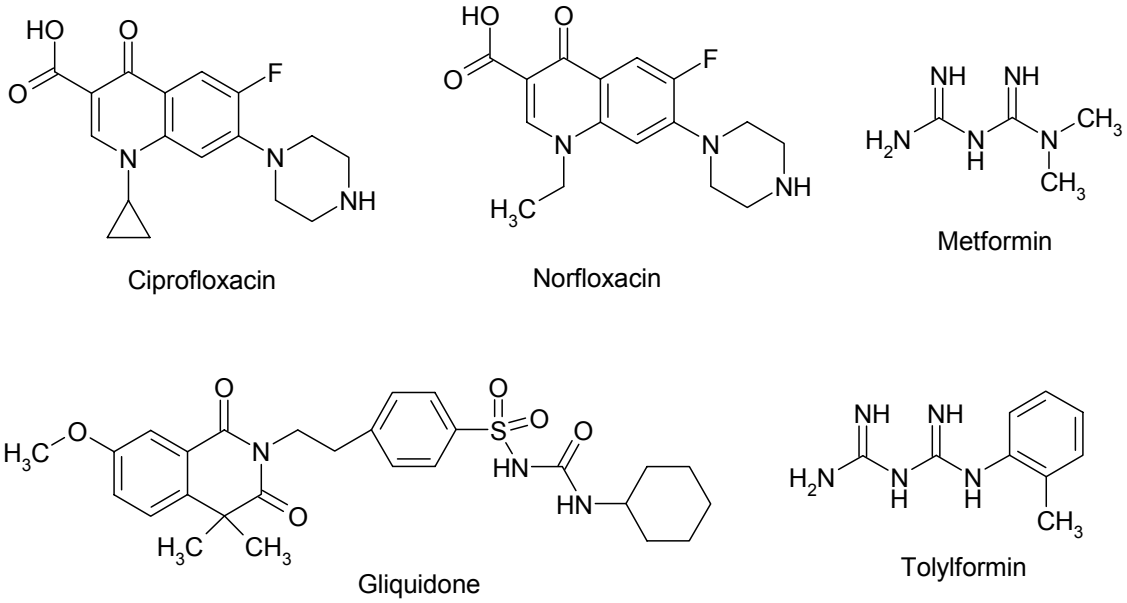

Fig. 1 – Chemical structures of studied compounds.

Table 1

Hydrophobicity parameters and acidity constants for studied compounds

Compound	log $K_{ow,}$(calculated)*	log $K_{ow,}$(experimental)
Metformin	-2.64	-
Norfloxacin	-0.31	-1.03
Ciprofloxacin	-0.0008	0.28
Tolylbiguanide	1.05	-
Gliquidone	4.65	-

* Calculated with EpiSuite Software (KOWWIN).[13]

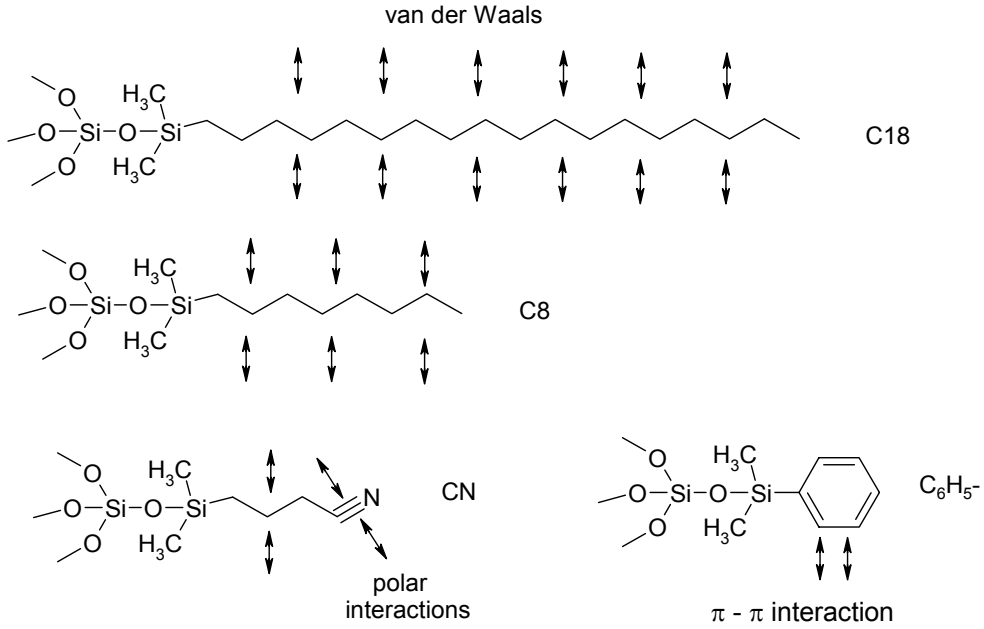

Fig. 2 – Chemical structures of the bonded silica based adsorbents used in this study.

Table 2

Characteristic properties of sorbents used in present SPE study

#	Cartridge type	Sorbent mass (mg)	Surface area (m^2/g)	Pore size (A)	Particle size (μm)	Carbon loading (%)
1	C18	200	541	60	45	25.7
2	C8	200	571	60	45	15.6
3	Phenyl	200	562	60	45	12.6
4	CN	200	-	60	50	4

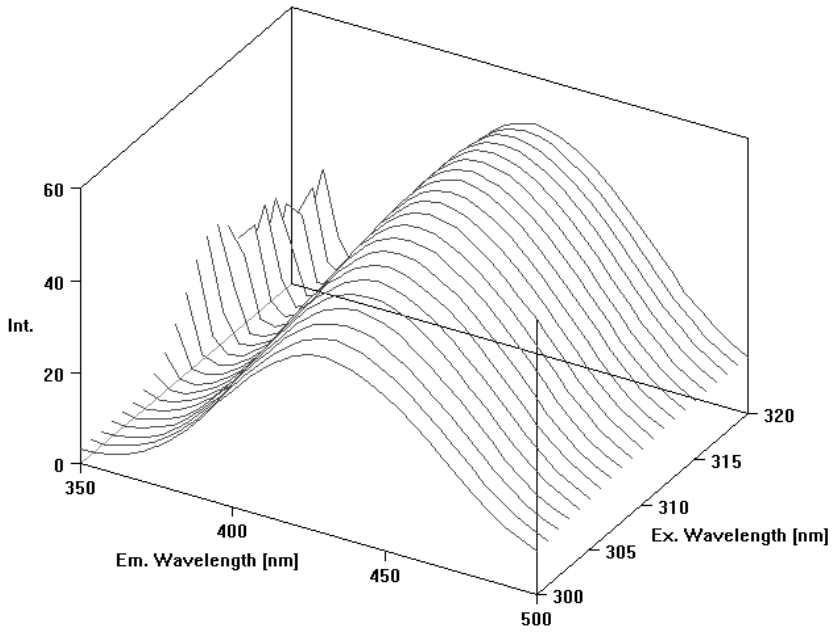

Fig. 3 – Fluorescence 3D spectrum of a solution containing 50 ng/mL gliquidone in water.

Equipments: An automated SPE HT 400E with autosampler was used in this study. The sorbent characteristics are presented in Table 2. The absorption spectra were recorded with a Jasco V-530 double beam spectrometer, in 1 cm quartz cells and the fluorescence spectra were recorded with a Jasco FP-6500 fluorometer. The absorbance were measured at

λ_{max} = 233 for metformin and λ_{max} = 236 nm for o-tolyl biguanide. The excitation wavelength for ciprofloxacin and norfloxacin were 275 nm, and 310 nm for gliquidone; the emission wavelength was 447 nm for ciprofloxacin, 443 nm for norfloxacin and 431 nm for gliquidone (its 3D spectrum is given in Fig. 3). All the experiment was performed at room temperature. The flow rate was kept constant during the experiments (1 mL/min).

RESULTS AND DISCUSSION

This study focused on the retention process of chosen compounds on adsorbents of a wide range of polarity and hydrophobicity, but meanwhile it took into consideration the possible applications of SPE to the isolation and concentration of studied compounds from various samples.[10] For this reason and for particular feature of the chosen compounds of absorbing or emitting UV-radiation, the studied samples were situated at concentrations of ppm or ppb levels, respectively.

Ciprofloxacin

This compound belongs to the class of fluoroquinolone antibiotics. Fluoroquinolones are molecules with zwitterionic behavior, as they have functional dissociable groups. Because of the acido-base properties of fluoroquinolones, solid phase extraction is expected to be strongly pH dependent.[14] Acidity constants of fluoroquinolones (pK_{a1} and pK_{a2} values) correspond to deprotonation of carboxylic acid group and protonation of piperazinyl amino group (N_4) of fluoroquinolones.[15] The elution curves for this compound on the four adsorbents used in this study are given in Fig. 4. This compound is retained efficiently on CN and C_6H_5 silica-based adsorbents due to the polar-polar interaction with cyano groups from adsorbent surface, while the phenyl functionality allows the highest interaction between analyte and adsorbent due to π - π stacking interactions. The model obtained with the aid of Marvin program shows some electrostatic charges that could interact with cyano group from the adsorbent. Some negative charges are located on the F atom, O atoms from carboxyl group and N atom from piperazine ring, which may interact with the positive charge from C atom of cyano group on the surface of the cyanopropyl silica adsorbent. High charges on the molecule of ciprofloxacin do not allow the van der Waals interactions with octadecyl chain. However, the breakthrough curve obtained for C8 adsorbent indicates a strong retention on octyl chains from the corresponding silica based adsorbents, which could be explained by lower hydrophobic character of C8 compared to C18 adsorbent. Therefore, this compound can not be adsorbed on the surface of strong hydrophobic adsorbents. According to the breakthrough curve obtained for C_6H_5- silica based adsorbent this compound can be adsorbed very efficiently due to the π - π stacking interactions between aromatic rings of ciprofloxacin and phenyl ring of the adsorbent.

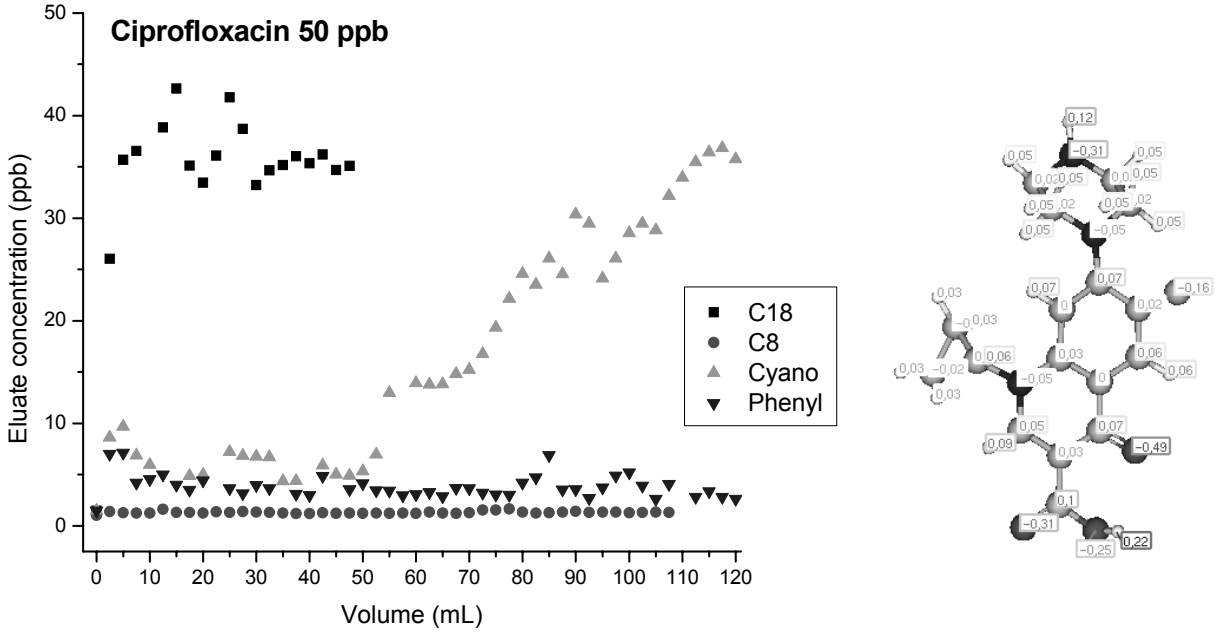

Fig. 4 – The breakthrough curves for ciprofloxacin on four adsorbents and its structure with charged atoms obtained by Marvin program.[16]

Norfloxacin

According to the data from Table 1 norfloxacin is more polar than ciprofloxacin. From this point of view we may expect to observe a poor adsorption of this compound on C18 silica based adsorbents and a stronger adsorption on CN adsorbent compared to the previous one. The breakthrough curves for norfloxacin obtained in the same conditions as for ciprofloxacin are shown in Fig. 5. They proved the less adsorption on C18 adsorbents in comparison to the previous studied fluoroquinolone. However, the strongest retention was obtained on phenyl silica adsorbent, and on C8 adsorbent, which is unusual for this type of analytes. The only explanation that could be given is related to the possible low inertness of the C8 bonded silica after derivatization and thus a high content of the silanol on its surface that could be involved in strong dipole-dipole interactions or hydrogen bonding with carboxyl and amino groups from norfloxacin.

Tolylbiguandine

Although this compound has a hydrophobic moiety (*i.e.* phenyl ring) it exhibited no affinity towards C18 adsorbent. Its curve was omitted from Fig. 6 owing to the fact that the breakthrough concentration of the eluate was the same with the solution percolating the cartridge. On the other hand, as can be seen from Fig. 6 the breakthrough curves of this studied compound were much closer to the theoretical shape and this observation could be an argument to the distinct forces involved into the retention process. Tolylbiguandine can interact, for instance, with phenyl rings from the adsorbent surface by means of π-π stacking interactions which are however of low intensity and thus the small value of breakthrough volume of the cartridge which resulted (about 10 mL of indicated solutions in Fig. 6). A small interaction can be deduced also from the breakthrough curve obtained for C8 silica adsorbent, which can be assigned to the hydrophobic interactions between tolyl moiety of analyte and C8 chains from adsorbent surface. This time the polar interactions between guanidine moiety of analyte and residual silanol groups from adsorbent surface are practically negligible owing to the strong interaction of guanidine moiety with water molecules from sample solvent.

A characteristic breakthrough curve was obtained for tolylbiguanidine for CN adsorbent, the shape indicating a two steps profile, which may be assumed to a multi-layers adsorption on the cyano sites from adsorbent surface.

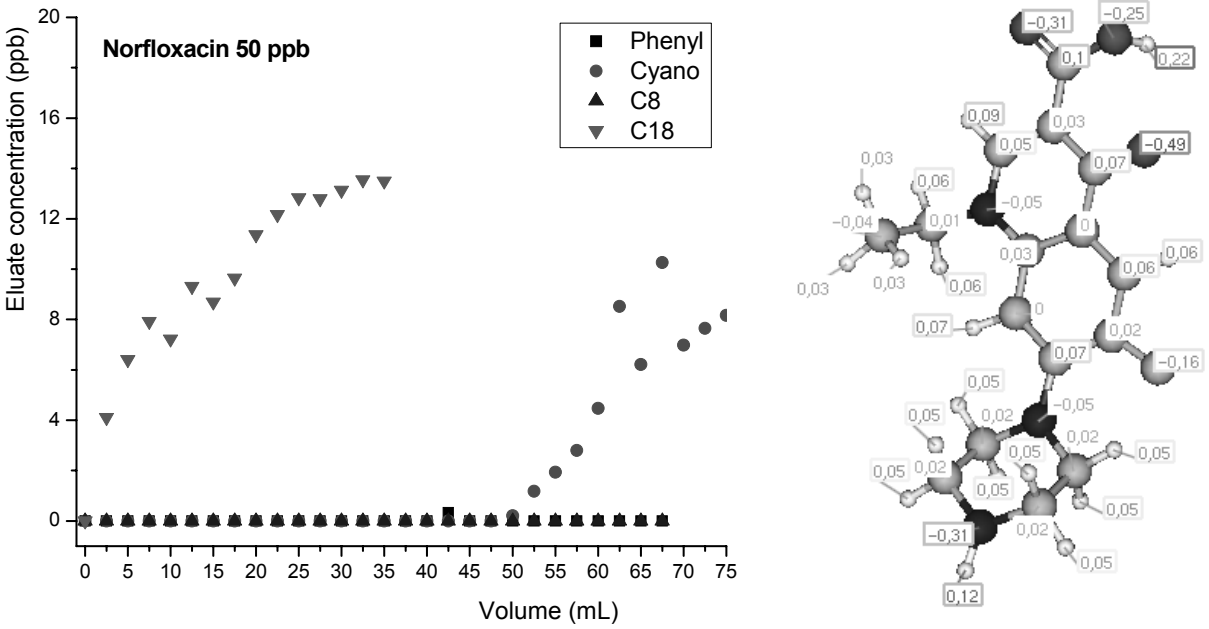

Fig. 5 – The breakthrough curves for norfloxacin on four adsorbents and its structure with charged atoms obtained by Marvin program.

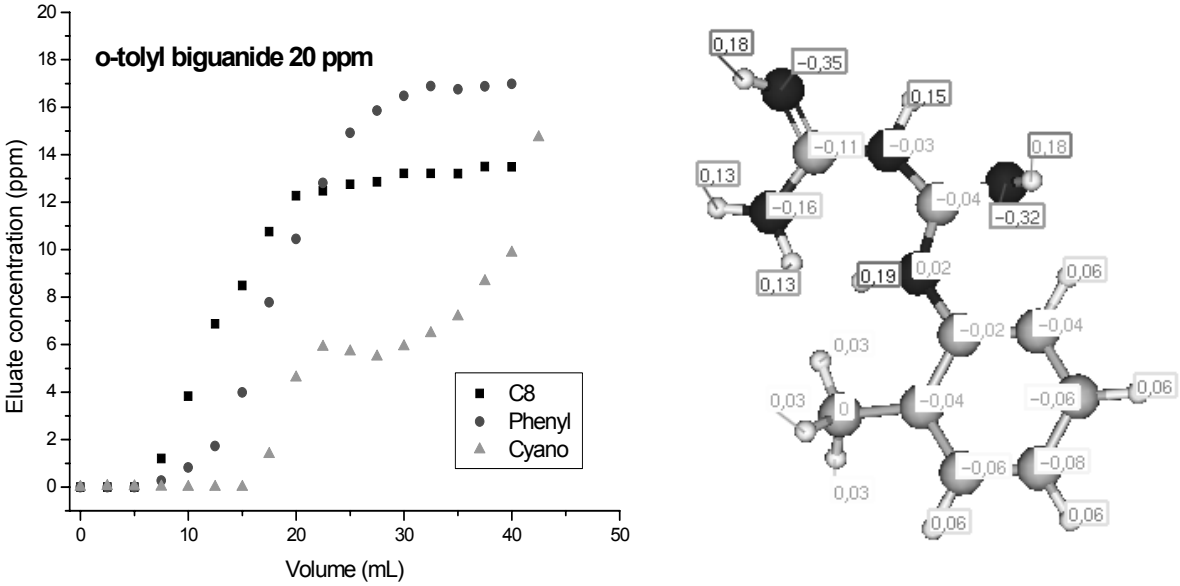

Fig. 6 – The breakthrough curves for tolylbiguanidine on three adsorbents and its structure with charged atoms obtained by Marvin program.

Dimethylbiguandine

Dimethylbiguanidine, known also as metformin, showed no retention towards C18 adsorbent when its concentration in eluate was identical to that from the solution applied to the cartridge (20 ppm, as indicated in Fig. 7). A slight interaction with C8 can be observed from this shape characterizing the breakthrough curve as depicted in Fig. 7. The higher polarity of phenyl adsorbent compared to C8 can be deduced from its elution curve, when the cartridge breakthrough occurs at a volume of about 5 mL solution of this analyte. On the other hand, the highest interaction was observed for CN adsorbent, although after reaching the hold-up volume of the curve a slight desorption prevailed over the adsorption process. The interaction of imino group from analyte molecule with cyano group from adsorbent was not however so strong to obtain a high retention, as has been proved from retention studies in HPLC with CN stationary phase.[17]

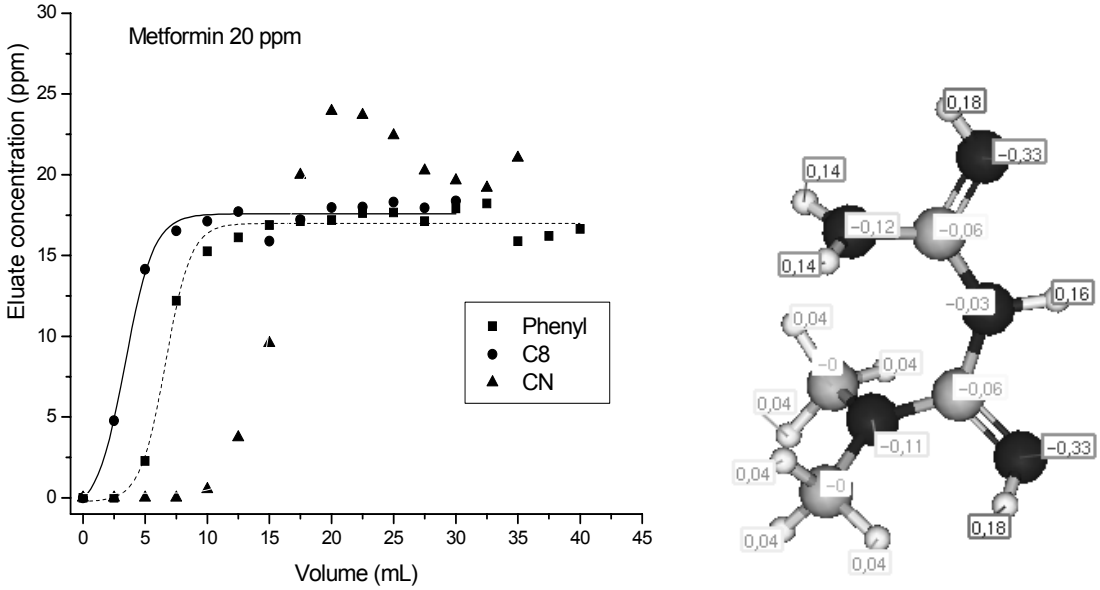

Fig. 7 – The breakthrough curves for dimethylbiguanidine on three adsorbents and its structure with charged atoms obtained by Marvin program.

Gliquidone

This compound has a high hydrophobicity (lg K_{ow} = 4.65, as predicted by fragment methodology). Its retention on C8, C18 and C_6H_5- adsorbents were so high that the cartridge breakthrough did not take place even after 100 mL of loaded solution. Due to its hydrophobic character it is likely that this compound not to interact with a polar adsorbent (omitted from the graph given in Fig. 8). Such supposition was demonstrated by its breakthrough curve on CN adsorbent, which showed a small value of the breakthrough volume (about 5 mL according to Fig. 8). Although gliquidone has some negative charges on its molecule (according to the model obtained with Marvin program) its interaction with polar groups from CN adsorbent surface seems not possible to occur. Its curve can be fitted by means of Boltzmann's function, as shown in Fig. 8.

Breakthrough parameters

The retention parameters (given in Table 3) can be estimated from its characteristic points describing the breakthrough curves, which have a sigmoidal shape (as can be seen in the above figures):[18]

V_B – the volume of the mobile phase, which corresponds to 1% of maximum concentration of the analyte in the effluent;

V_M – the hold-up volume, the volume of the mobile phase that corresponds to 99% of the maximum concentration in the effluent;

V_R – the retention volume of the model solute, which corresponds to the inflection point of the curve, where the solute adsorption is in equilibrium with desorption from sorbent surface.

These values have been obtained from the experimental curves which allowed their fitting by means of Boltzmann's function and calculated with equations given in ref.[14,18]

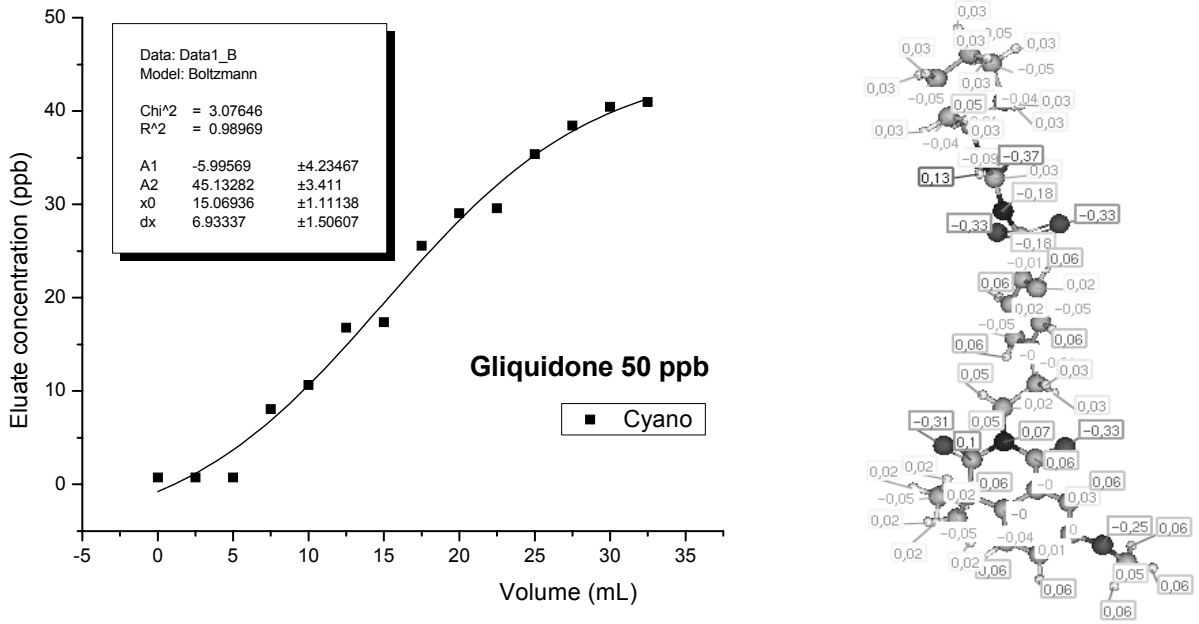

Fig. 8 – The breakthrough curves for gliquidone on cyano adsorbent and its structure with charged atoms obtained by Marvin program.

Table 3

Breakthrough parameters resulted from percolating the cartridges with individual solutions (indicated in *Experimental*)

Compounds	Cartridge type	V_B (mL)	V_R (mL)	V_M (mL)
Metfomin	C8	0.3	3.4	8.7
	Phenyl	2.9	6.7	11.3
o-Tolyl biguanide	C8	3.8	12.5	27
	Phenyl	6.6	18.4	33.2
Gliquidone	CN	1.7	15.1	47.8

CONCLUSIONS

The retention study of five multi-functional compounds of different hydrophobicity on four silica based adsorbents of different polarity revealed the complexity of the adsorption process of these compounds. The sigmoidal-like shape predicted by theory was obtained only for some cases, when the retention can be governed by one driving force, such as hydrophobic interactions, or π-π stacking. In case of cyano adsorbent that has polar characteristics the shapes of the breakthrough were like a double sigmoidal allure, which could be explained by the possibility of adsorption of one or more analyte molecules on the adsorption sites given by cyanopropyl moiety of the adsorbent. Molecular modeling of the studied molecules with the aid of Marvin program revealed the charged centers on these molecules, which can be involved in dipole-dipole interactions with adsorbent (cyano group, or residual silanol from silica surface). The more number of charged centers the less possibility of interacting between hydrophobic surface and analyte molecule – a conclusion that has been drawn from the breakthrough curves of all chosen compounds, excepting gliquidone, which is a very hydrophobic compound. Multi-functional structures lead to a random distribution of the points on the breakthrough curve as has been proved for two fluoroquinolones or biguanidines, which can be involved in more than one single intermolecular interaction.

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