POSTER SESSION I WEDNESDAY, JUNE 8 th, 2011

CHAIRPERSONS: T. Kowalska and Ž. Tešić

1.

Mapping fragmental drug-likeness in the MoStBioDat environment: intramolecular hydrogen bonding motifs in β-Ketoenols

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The general knowledge of hydrogen bonding effects is extremely important in chemistry, in particular, in medicinal chemistry and drug design, where molecular recognition and binding between a small molecule and a target receptor is decided by short-range noncovalent interactions. Intramolecular hydrogen bonding in β-ketoenol, which is a tautomeric form of β-diketones, is an interesting example of the equilibrium increasing the stability of the enolized form significantly. The enolizing motif (O=C–C=C–OH) is a structural moiety that is fairly common in chemically and structurally diverse sets of molecules that has recently been identified as a promising pharmacophoric pattern, e.g. indicating a potent anti-HIV-integrase activity.

This bidentate, oxygen-based difunctional building (sub)block might be involved in a variety of molecular effects involving binding divalent metal ions, hydrogen-bond acceptors (HBA) and hydrogen-bond donors (HBD). The metal chelating ability of the system might play a crucial role in the antiviral activity against HIV, blocking the integration step. Moreover, the spatial arrangement of the carbonyl and hydroxyl groups seems to determine the capability of β-ketoenol derivatives to recognize the surrounding environment by forming inter- and intra-molecular hydrogen bonds (IHB) determining the antiviral activity of the compounds.

In recent years chemoinformatics has seen an explosion in available molecular information resources. At the same time the investigations of the hydrogen bonding motifs provide a challenging problem. Although databases are frequently *on line* and supported by a searching capability, this option is not always useful if we are to precisely define the complex molecular fragments or substructures to be screened. Thus, we have recently developed a novel molecular and structural database managing system (MoStBioDat), which is available as a public domain package designed as a dual purpose storage/extraction platform that maintains high-standards of data integrity and reliability and provides software-based solutions for massive *in silico* protocols.

In our last work we report the practical application of the system for a systematic survey of the intramolecular hydrogen bonded (IHB) motifs in β-ketoenol derivatives. The virtual 3D data derived from the ZINC and PubChem repositories have been compared to the experimentally determined CSD results that acted as a ‘benchmark’ database. Differences specific for each database were discovered, which indicated inaccuracies in the simulated data.

2.

Chemometric models for efficient predictions of chromatographic behavior of ziprasidone and its impurities

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The retention behavior of ziprasidone and its five main impurities was investigated by use of the RP–HPLC. The optimal chromatographic conditions (gradient elution mode with mobile phase consists of water phase - 1% TEA in 0.05M potassium dihydrogen phosphate solution, pH adjusted to 2.5 by orthophosphoric acid, and organic phase – acetonitrile, working temperature of 25°C, UV detection at 250 nm, the flow rate of 1.5 ml/min and run time of 20 minutes) were applied to examine 20 different reversed-phase columns for their selectivity and efficiency towards the ziprasidone and its five impurities. Influences of the different stationary phases on the retention parameters and selection of the most suitable columns were performed by means of principal component analysis. The same elution order was observed for the different components with most of the columns examined. Most of these columns did not allow separation of critical pair ziprasidone and impurity II. However, separation of this pair was achieved on three columns, and the optimal column (Waters Spherisorb® ODS 1, (4.6mm×250 mm, particle size 5.0 μm)) was selected. Finally, relationship between molecular parameters and chromatographic behaviour of the ziprasidone and its five impurities was examined.

3.

Knowledge discovery in molecular and structural chemical databases

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Knowledge Discovery process, also known as Knowledge Discovery in Databases (KDD), is defined as the nontrivial extraction of implicit, previously unknown, and potentially useful information from data.[1] It is the most distinguished branch of *data mining* and provides accurate detailed data description. Furthermore, there is an urgent need for invention and development of computational approaches to extract reliable helpful information from all kind of databases, especially the rapidly growing molecular and structural chemical data resources.

Successful modeling work is relative to the availability of essential structural and experimental data, and yet a number of molecular databases for available organic compounds, screening compounds, medicinal agents (drugs), as well as databases with ADMET properties and physico-chemical properties are publicly available and can be used in drug design, e.g. PubChem Compound, ZINC, ChemDB, ChemBank, ChEMBL and DrugBank databases contain ca. 32, 13, 4.1, 1.2, 0.76 and 0.04 mln compounds, respectively.

Here we report an application of a novel and unique molecular and structural database managing system, MoStBioDat[2] for the massive *in silico* protocols parallely analyzing small molecule ligand and protein data. In this study, a compilation of various publicly available databases of small molecules has been analyzed to locate all possible occurrences of the intramolecular hydrogen bonded motifs in catechols.[3] The comparison of the experimentally determined structural data to those that are simulated using virtual structural data indicated a high uncertainty of the topology of this system for *in silico* simulations using data coming from different sources.

What is more, mining small molecule databases relevant to drug discovery could be also a fruitful method for classifying chemical compounds as being druglike and/or leadlike. In some case it is feasible to identify common molecular fragments, so-called *privileged motifs*, which ease ligand binding to an individual receptor or particular receptor family.[4] As a result, privileged scaffolds might be successfully applied in drug discovery process, e.g. as core structures for synthesis[5] and optimal starting points for the library design.[6] Although privileged substructures are intended to be target class-specific it has been shown that this separated molecular subunits also appeared in compounds active against other target families.[7] Furthermore, a single separated structural subunit time and again could be present in thousands substances including various natural products exhibiting miscellaneous pharmacological activities. Frequency of occurrences of that kind generic druglike molecular fragment among drug populations and bioactive compounds ensembles could be a valuable index of privileged structures estimation. By screening databases we can estimate the population of privileged (sub)structural motifs[8] or investigate the evolution of organic chemistry which has a well-defined, modular architecture.[9] This forced us to perform comprehensive exploration of azanaphthalene polypharmacology.

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4.

The influence of heat effect on the peak profiles in overload conditions

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The trend in modern chromatography, still tends towards the achievement of high efficiency and shorter analysis times. To achieve the high column efficiency and the short analysis times, the use of columns packed with sub-2μm particles, are necessary. However, the requirement of the shortest time of analysis enforces the application of high mobile phase velocity, which causes a large value of head pressure to overcome the viscous forces. For 1.7 [μm] particle diameter used in Very High Pressure Liquid Chromatography (VHPLC), the required pressure can be more than 1000 bar. In columns, operating at high mobile phase velocities, under high pressure gradients, a large amount of heat due to the viscous friction of the eluent percolating through the column bed, is produced. The heat generation, cause the formation of an axial and a radial temperature gradient, which follows in a loss of column efficiency. In many papers, the influence of heat generated by viscous friction on the column efficiency was analysed for linear isotherm equation, but not for nonlinear.

We have measured the retention time of butyric acid for three different case of heat transport: the natural convection, the air thermostated column and the column thermostated in a water bath. The Dionex RSLC Polar Advantage II (PA2) 100mm x 2.1mm column packed with 2.2 [μm] totally porous particle was used. The aim of this work was to present the results obtain in overload conditions.

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5.

Chiral separation in normal-phase liquid chromatography: Enantioselectivity of recent polysaccharide-based selectors Part II. Enantioselectivity at optimization conditions

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Abstract

Enantiomers of a chiral drug molecule may show significant differences in their pharmacological, pharmacokinetic or toxicological effect in biological systems [1]. Therefore, enantiomeric separation of chiral compounds is a critical challenge in drug discovery and development. Separation of enantiomers can be achieved using different chromatographic techniques such as gas chromatography (GC), liquid chromatography (LC), supercritical fluid chromatography (SFC), and electromigration techniques. To avoid time-consuming trial-and-error approaches for developing a chiral separation method, generic strategies, consisting of a screening and optimization stages, have been developed. Earlier, a set of pharmaceuticals with different chemical structures has been used to evaluate the enantioselectivity of four recently commercialized polysaccharide-based chiral stationary phases, Lux Cellulose-1, Lux Cellulose-2, Lux Amylose-2 and Lux Cellulose-4 and of three earlier commercialized columns, Chiralpak AD-H, Chiralcel OD-H and Chiralcel OJ-H, using the screening conditions of an existing generic separation strategy in normal-phase liquid chromatography (NPLC) [2]. In the current study, the applicability of previously developed optimization steps [3] on those columns was examined using 48 drugs (74 optimization cases). The resolution, peak shape and the analysis time were nicely improved after the application of the original optimization, for 49/74 cases (66%), baseline resolution was observed. The introduction of some modifications to the original optimization increased the number from 49 to 62 cases, i.e. from 66% to 84%. Finally, an updated generic separation strategy in NPLC was proposed.

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6.

Evaluation of reduced test sets for the development of separation strategies for chiral drug compounds

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During the past decades, a lot of emphasis is put on chiral analysis in pharmaceutical research. Chiral lead compounds have to be analysed in such a way that both, the therapeutically active enantiomer (eutomer) as well as the other (distomer) are identified, quantified and well characterised since in biological environment eutomer and distomer behave differently with possible harmful effects of the distomer as a consequence.
Therefore, the development of chiral separation strategies gained a lot of attention during the past decades. These strategies are used in different stages of chiral drug development. The chiral recognition mechanisms are still not always completely elucidated and there are no universal selectors available to obtain separation between all eutomers and distomers. The defined strategies might be updated when new selectors, claimed to have a broader enantioselectivity than previous ones, are marketed. These updates as well as the development of such strategies are time consuming, especially when large test sets are used.
The goal of this study is to define smaller test sets and verify whether it is still possible to select the most enantioselective systems with these sets. For the selection of the reduced test sets the molecules were described by molecular descriptors. These descriptors 'translate' the chemical information of a molecule into mathematical information (numbers). This will allow selecting a predefined percentage of the original test set by running a selection algorithm (Kennard and Stone) on the data.
The results showed that even with a decrease of 70% of the original test set (19 instead of 62 compounds), the as most enantioselective systems selected ones, turned out to be mainly the same as those obtained with the large test set.

7.

Updating a screening strategy for chiral separations in supercritical fluid chromatography with new chlorinated polysaccharide-based selectors

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Chiral separations are an extensively studied topic, especially in pharmaceutical analysis as drug enantiomers potentially exhibit different properties in the human body.
High-pressure liquid chromatography in RPLC, NPLC and POSC modes remain the most widely used techniques for chiral separations in the pharmaceutical industry. However, drawbacks are related to these techniques, such as the rather long analysis times that limit the throughput and/or the high consumption of toxic and flammable solvents.
Therefore, supercritical fluid chromatography (SFC) has gained interest as an alternative. SFC offers the benefit that higher flow rates can be used in comparison with conventional HPLC, thus reducing column-equilibration- and analysis times and enabling a higher throughput. Additionally, SFC methods have a lower consumption of organic solvents and can thus be considered more environmental friendly.
To enable fast chiral method development, generic chiral separation strategies are defined. A first step in these strategies is a screening step. The aim of these screenings is to quickly determine whether an (acceptable) separation of a certain racemate can be achieved on a given chromatographic system. A generic screening step for SFC has been defined earlier by Maftouh et al [1]. Recently, new chiral stationary phases (CSP) have been introduced containing chlorinated polysaccharide-derivatives as chiral selectors. These new CSP have proven to display an even broader enantioselectivity in NPLC and POSC than those used in the initially earlier defined screening steps.
In this study, the previously defined SFC screening conditions were applied on new chlorinated polysaccharide-derivatives. New mobile phases, containing both a basic and an acidic additive, are tested with the aim of selecting the most generic enantioselective screening conditions. The global result of this study is on updated generic screening step, with a broader enantioselectivity and a faster analysis. Hereby a higher success rate for chiral separations is achieved.

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8.

The influence of sample matrix composition and injected sample volume on the electrophoretic behaviour of small non-enveloped viruses

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For the past three years the authors have been involved in the development of capillary electrophoresis (CE) methods for poliovirus (PV) identification and separation from subviral particles in various types of samples. Based on the PV concentration and viral purity of the sample, the samples were injected in plugs of 1%, 5% and 12% effective capillary length to obtain an optimal separation and an acceptable signal level. Various samples, with diverse matrix composition or purities were successfully separated, but a certain matrix influence was observed.

The present study thouroughly investigates the electrophoretic behavior of poliovirus injected as plugs of 1%, 5% and 12% effective capillary length, respectively, to understand the CE limits and benefits when separating virus suspensions. When samples are injected as 5% and 12% plugs, the signal is enhanced. However, the complexity of the sample did not allow a straightforward identification of the signal enhancing mechanism. The effect of sample matrix, temperature, buffer or SDS concentration were carefully considered.

The matrix composition proved to be especially important for the signal increase and virus stability. The sample matrix had only a limited effect on both PV and electroosomotic flow (EOF) mobility. When larger sample plugs were injected, the PV mobility slowly increased. A decrease with 4°C of the separation temperature or the doubling of the BGE concentration decreased EOF almost ten times more than increasing the injected sample from 1% to 12% plug. However, extremely diluted samples injected as 12% plugs induced a serious EOF increase. In case of PV, the decrease in the separation temperature with 6°C reduced the PV mobility with 30%.

The results provide a better understanding of CE separation of non-enveloped viruses, opening the way to further application, such as stability indication, detection of subviral particles or the study of the interactions between poliovirus and nanobodies, RNA or cellular proteins.

9.

Inverse gas chromatography in the examination of modern organic-inorganic hybrid materials

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In the last years, research on a new class of compounds such as hybrid materials are becoming very popular. Thanks to the ability to control materials properties within the wide range hybrids can be applied in various fields as nanoelectronics, biomaterials, pharmacy and many others.

Inverse Gas Chromatography (IGC) is the method allowing to examine the physicochemical properties of various materials including multicomponet hybrid systems. IGC is an extension of conventional gas chromatography. In this method the examined material is placed in the chromatographic column and its properties are concluded basing on retention behavior of carefully selected test compounds. Acid-base and dispersive properties of the surface may be studied by means of IGC.

The experiments carried out allowed to examine the surface properties of individual components of the hybrids, as well as these for two-and three-component systems. This enabled the evaluation of the mutual impact magnitude of the hybrid material components.

10.

The HPLC study of aqueous and non-aqueous solutions of phenylalanine and phenylglycine

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Among chiral compounds investigated within the framework of our research project (profens, amino acids, hydroxy acids), phenylalanine plays a particular role due to its recognized biological importance, whereas phenylglycine is an important substrate to produce drugs and cosmetics.

In our earlier studies [1-4], we described the results of investigating the remarkable phenomenon of spontaneous *in vitro* oscillatory chiral conversion of the selected optically pure amino acids (e.g., *L*-phenylalanine, *L*-alanine, *L*-tyrosine, *L*- and *D*-phenylglicine), when dissolved in the abiotic aqueous and non-aqueous media, and then aged for certain periods of time at ambient temperature, in the stoppered glass vials. These investigations were carried out with aid of the chiral thin-layer chromatography and polarimetry. Later, we managed to experimentally prove that the spontaneous *in vitro* oscillatory chiral conversion of *L*- and *D*-phenylglycine is accompanied by the spontaneous oscillatory polycondensation of these compounds and our polycondensation results originated from the non-chiral high-performance liquid chromatography [5].

High-performance liquid chromatography with the diode array detection (HPLC-DAD) is a reliable and accurate enough tool to monitor and quantify oscillatory reactions in purely organic and colorless solutions, basically with the UV-absorbing analytes. In this study, we employed HPLC with the two different detectors (diode array (DAD) and evaporative light scattering (ELSD)) to present the chromatographic results of chemical transformation with *L*-phenylalanine and *L*-phenylglycine, when dissolved in 70% aqueous ethanol and in pure dichlomethane, and then stored for certain periods of time in the stoppered glass vials at ambient temperature.

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11.

Quality control of *citri reticulatae pericarpium*: exploratory analysis and discrimination

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Traditional medicine (TM) is becoming a more popular approach to treat or prevent diseases in many countries, including our Western society, making it a lucrative business worth billions of dollars. The main problem in this booming industry is the lack of quality control, needed to ensure the products identity and quality, and the patient's safety.

An example of TM is the dried peel from the mature fruits of *Citrus reticulatae Blanco* (PCR) and its cultivars. This herbal product, also called *Chen Pi*, is often used in traditional Chinese medicine to eliminate phlegm and strengthen the spleen. While the Chinese Health Department only requires *Citri reticulatae* pericarpium samples to contain at least 3.5% of hesperidine, most citrus species meet this criterion. Other problems include the existence of '*mixed peels*' and '*coupled herbs*': i.e. contamination with other citrus species and other herbal parts of the same plant species, respectively.

To detect adulterations and contaminations, an HPLC methodology was developed for the fingerprint analysis of citrus samples. The data set was recorded in three different stages considering: (1) samples obtained as *Citrus reticulatae* pericarpium, (2) potential mixed peels and coupled herbs samples, and (3) dried peels of other citrus species. In a first data analysis step, an exploratory analysis was performed on the developed data sets using Principal Component Analysis. For the samples bought as *Citrus reticulatae* pericarpium, the PC1-PC2 score plot revealed two clusters based on the sample preparation procedure: a cluster of samples obtained in ground form and extracted as they were and a cluster of samples obtained as pieces of peel and extracted after grinding ourselves. To eliminate the variation between the fingerprints not caused by differences in species, the sample preparation procedure was re-optimized and the new PC1-PC2 score plot did not separate both groups anymore.

Once the HPLC optimizations were performed and the method validated, discrimination between the authentic PCR samples and all other samples was performed by probabilistic Discriminant Partial Least Squares. The established model was able to differentiate between both classes with a high reliability for each sample. Furthermore, evaluation of the score and loading plots of the model indicated nobiletin, tangeretin, naringin and hesperidin as important markers for the quality control of *Citrus reticulatae*

12.

Study of condensation oscillations with *L*-lactic acid in pure acetonitrile and aqueous ethanol

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Spontaneous nonlinear chemical processes that occur in the Nature still seem not to be adequately explored. On the other hand, these very processes can play crucial role in various different metabolic and evolutionary pathways. Tracing oscillatory reactions in purely organic and colorless solutions is a challenging experimental task. High-performance liquid chromatography with the diode array detection (HPLC-DAD) is a reliable and accurate enough tool to monitor and quantify such phenomena, basically with the UV-absorbing analytes. A bottleneck of the chromatographic analysis is, however, the time needed for a single analytical run, which makes continuous measurements of the concentration changes (needed for the kinetic assessment of the investigated reactions) virtually impossible, even if an autosampling device is under the hand. One way to circumvent this acute inconvenience is to obtain the shortest possible single analytical run, in that way getting a series of quantitative results bearing a semi-continuous importance.

Among chiral compounds investigated within the framework of our research project, *L*-lactic acid plays a particular role due to its known biological importance, but there has been no experimental evidence prior to our own research [1,2] on its ability to undergo a spontaneous oscillatory *in vitro* chiral conversion. One reason is that lactic acid is poorly retained in the HPLC systems [3] and in that way it causes considerable analytical problems. To solve these problems, an alternative enantioseparation method had to be developed [4] and it is probably noteworthy that one thin-layer chromatographic method is also available [5].

In this study, we present high-performance liquid chromatographic and mass spectroscopic results on chemical transformation of *L*-lactic acid, when dissolved both in 70% aqueous ethanol and in pure acetonitrile, and then stored for certain periods of time in the stoppered glass vials at an ambient temperature. In order to gain a better insight in the nature of the investigated processes, in our experiments we employed HPLC with the three different detectors (diode array (DAD), evaporative light scattering (ELSD), and mass spectrometric (MS) detector).

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13.

The HPLC and optical tracing of the molecular level inhomogeneity with the aqueous ethanol solution of *S*(+)-naproxen

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Discovery of chemical processes running in abiotic systems according to non-linear dynamics and then tracing the kinetics thereof is not a simple experimental task. If the non-linear processes run in the colorless organic solutions (thus escaping the straightforward visual inspection), then the most reliable measuring techniques probably are the chromatographic ones.

In our earlier studies [1-3], we described the results of investigating the remarkable phenomenon of spontaneous *in vitro* oscillatory chiral conversion of the selected optically pure profen drugs (e.g., *S*(+)-ibuprofen, *S*(+)-naproxen, *S*(+)-ketoprofen, *S*(+)-flurbiprofen, and *R*(-)-flurbiprofen), when dissolved in the abiotic aqueous and non-aqueous media and then aged for certain periods of time at ambient temperature, in the stoppered glass vials. These investigations were carried out with aid of the chiral thin-layer chromatography and polarimetry.

Later, we managed to experimentally prove that the spontaneous *in vitro* oscillatory chiral conversion of the low-molecular-weight carboxylic acids is accompanied by the spontaneous oscillatory polycondensation of these compounds and our polycondensation results originated from the non-chiral high-performance liquid chromatography [4,5].

In this study, we present the results of a simple yet more advanced experiment carried out with *S*(+)-naproxen dissolved in 70% aqueous ethanol, then placed on Petri dishes in an optical zooming scanner, and scanned in UV light at 254 nm, in the selected time intervals for ca. four hours. The employed technique was aimed to highlight the dynamic aspect of the discussed supramolecular self-organization of the investigated profen drug solution in a given period of time and to demonstrate the fluctuating density inhomogeneity thereof. The sequence of the snapshots obtained with use of the zooming scanner was discussed in the context of the obtained high-performance liquid chromatographic data.

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14.

On the influence of impregnation with *L*- and *rac*-arginine on retention of naproxen in the thin-layer chromatographic systems

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Silica gel impregnated with *L*-arginine was for the first time used to enantioseparate the racemic ibuprofen mixture in 1996 [1]. In order to better understand the mechanisms governing profen enantioseparation and to further explore the potential of *L*-arginine for this particular purpose, extensive investigations have been carried out with other profens (e.g., with naproxen), and comparisons were made between the performance of the impregnated and the non-impregnated silica gel layers [2-5]. Upon the results (i.e., densitograms and videoscans) obtained, it was assumed that the crystalline chirality of the silica gel layers enables partial enantioseparation of profen antimers in the direction perpendicular to that of the mobile phase flow, whereas the molecular chirality of *L*-arginine is responsible for enantioseparation parallel to this direction [6].

In this study, we present a further and important step in the aforementioned research, which depends on a comparison of the influence exerted by impregnation of the silica gel and the silica gel – kieselguhr layers with *L*-arginine and *rac*-arginine on the retention of the selected test enantiomers. To this effect, we used 70% aqueous solutions of *S*-(+)-naproxen and *rac*-naproxen as the test solutions and six different stationary phases, listed below:

- silica gel impregnated with *L*-arginine,
- silica gel impregnated with *rac*-arginine,
- non-impregnated silica gel,
- silica gel – kieselguhr mixture impregnated with *L*-arginine,
- silica gel – kieselguhr mixture impregnated with *rac*-arginine,
- non-impregnated silica gel – kieselguhr mixture.

A comparison of the chromatograms obtained on silica gel impregnated with *L*-arginine and *DL*-arginine confirmed our earlier findings on the influence of the molecular chirality of the impregnating agent on the retention and enantioseparation of naproxen. A comparison of the chromatograms obtained on the silica gel and the silica gel – kieselguhr mixture emphasized the influence of the adsorbent's chromatographic activity on the retention of the test samples.

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