POSTER SESSION II

THURSDAY, MAY 25th, 2017

CHAIRPERSONS:

Kamilla Acs and Biljana Otašević

1.

Chromatographic determination of phenolic compounds in comercial samples of *Cistus incanus* L

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Phytotherapy is becoming very popular and increasingly common [1] and among the herbs, *Cistus* species has gained great popularity because of a wide spectrum of activity (e.g. antibacterial, antioxidant, antiviral and antifungal activity) [2,3]. Among the chemical compounds which determine the pharmacological properties of *Cistus incanus L.*, polyphenol compounds are the most significant.

The aim of this study is comparison of the phenolic fraction of tested comercial samples within one species of the herb originating from different manufacturers. Samples of each plant were macerated and subjected to exhaustive extraction in the Soxhlet apparatus. The herb extracts prepared in that way were subjected to a multistage extraction allowing for the isolation of individual fractions of phenolic compounds, including flavonoid aglycones, free phenolic acids and flavonoid glycosides. Each fraction was then analyzed by means of TLC chromatography.

Tested in our research commercially available samples showed significant differences in the composition of the phenolic fraction. The obvious conclusion is that the composition of herbally based preparations can be quite variable. Various samples of *Cistus incanus* L. have a different polyphenol profile which may involve their different health benefits. Thus, proper standardization the chemial composition and quality control of raw materials and the herbal products should be carried out.

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2.

Development and validation of RP HPLC method for determination of cyanocobalamin and phenole in pharmaceutical dosage form

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In this paper, development and validation of RP HPLC method for determination of cyanocobalamin (B12), water-soluble vitamin and antioxidant phenol is presented. The analysis was performed using column C8 250 mm x 4.6 mm, 5 μm particle size. Mobile phase was mixture of water and methanole (75 : 25 v/v). Column temperature was 30°C, mobile phase flow rate 1.5 ml/min and detection wavelength 361 nm for vitamin and 270 nm for phenol. The method was validated according to ICH Q2(R1) requirements. It was proved that this method is selective for determination of B12 and phenol. Linearity was confirmed with r values (r = 0,9994 for B12; r = 0,9992 for phenol). Accuracy was tested at three concentrations levels (80%, 100% and 120%) and confirmed by calculated recovery values (98.56 – 99.72% for B12; 99.07 – 101.39% for phenol). Precision was tested at two levels: intra-assay precision and intermediate precision. Calculated relative standard deviations were 0.92% and 0.18%, respectively. Small variations of mobile phase composition (organic solvent), column temperature and flow rate did not affect qualitative and quantitative system responses significantly, which proved method's robustness. Applicability of the method in routine was confirmed by analysis of commercialy available injecton for veterinary use.

Keywords: cyanocobalamin, phenol, RP-HPLC, ICH

3.

Analysis of retention behavior of selected antipsychotics and their impurities by thin layer chromatography

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Retention behavior and lipophilicity of aripiprazole and its nine impurities as well as ziprasidone and its five impurities have been examined by thin layer chromatography using RP-18 stationary phase and different mixtures of methanol, water and ammonia; and ethanol, water and ammonia as mobile phases. In both examined chromatographic systems linear relationships were established between retention parameters and the volume fraction of the methanol/ethanol in mobile phase ($r>0.948$ for methanol and $r>0.971$ for ethanol). Something higher correlation between determined hydrophobic parameters R_M^0 and calculated logP values was observed for methanol-water-ammonia/RP-18 ($r= 0.939$) compared to the ethanol-water-ammonia/RP-18 ($r=0.913$) chromatographic system. Also, retention parameters obtained when methanol was used as organic modifier showed higher values compared to the ethanol as organic modifier in the mobile phase. Experimentally obtained R_M^0 values and computed molecular parameters of the examined compounds were further used for the quantitative structure–retention relationship (QSRR) study in order to determine the most important properties governing retention. The QSRR modeling was performed with use of the partial least squares regression, and predictive performances of the developed QSRR models were tested by use of the cross-validation and external test set prediction. The obtained results revealed that apart from lipophilicity, topological descriptors and molecular weight of the tested compounds has the strongest influence on the retention behavior of examined antipsychotics and their impurities in the reverse phase thin layer chromatography. The predictive performance of the created QSRR model suggests its applicability for a reliable prediction of the retention behavior of the congeners.

4.

Characterization of unknown impurity of ziprasidone with new UPLC MS/MS method

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Ziprasidone is chloro-indolone class of an atypical or second-generation antipsychotic drug effective in the treatment of positive, negative and affective symptoms of schizophrenia showing a low propensity for extrapyramidal symptoms side effects, cognitive deficits and almost no effect on weight, glucose, lipid and prolactin levels [1].
With previously developed HPLC method for analysis of ziprasidone and its five main impurities (I-V) by our research group [2] was detected and successfully separated one novel unknown impurity (t_R 11.270 min) in the test solution, after 24 hours stored at the room temperature [3]. Chemical characterization of the unknown impurity of ziprasidone is essential for defining genotoxic potential of the compound and consequently establishes the quality, safety and efficacy of the drug substance [4]. Thus, a new highly sensitive and rapid UHPLC-MS/MS method was developed for qualitative and quantitative assay of the ziprasidone and its six impurities in raw material and pharmaceuticals and at the same time with possibility for characterization of unknown impurities. All seven analytes were eluted within the 7 min run time. The method was used for the detection and characterization of a new impurity of an unknown structure. The best separation was obtained on the Acquity UPLC BEH C18 (50 mm x 2.1 mm x 1.7 μm) column with mobile phase consisted of 10 mM ammonium-formate aqueous solution, pH = 4,7 adjusted with formic acid and acetonitrile, with a timed gradient mode and the flow rate of 0.3 mL/min and at the column temperature of 30°C. UHPLC–MS/MS analyses were carried out on a UHPLC–MS/MS system coupled to a triple quad Mass spectrometer with a heated electrospray ionization (HESI) interface in a positive-ion, except for the impurity IV in a negative-ion mode. The MS/MS fragmentation conditions were optimized individually for each compound in order to obtain both specific fragments and high signal intensity. Both calibration and sample data were obtained by selective reaction monitoring acquisition. Collision-induced dissociation mass spectra of known compounds, ziprasidone and impurities I-V were obtained by flow injection analysis (FIA). All compounds were identified and their masses were measured. The mechanisms of fragmentation of ziprasidone, impurities I-V and unknown impurity were proposed. Thus, with regard to the peak at *m/z* 823, HESI-spectrum, and the proposed fragmentation mechanism, the most probable structure of unknown impurity was presented.

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5.

Metabolite profiling of MAO-A inhibitor – moclobemide with the use of human liver microsomes and LC-MS method

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Moclobemide (4-chloro-N-(2-morpholin-4-ylethyl)benzamide) is a reversible and highly selective inhibitor of monoamine oxidase A (MAO-A). Moclobemide increases the content of serotonin, noradrenaline and dopamine in the brain and decreases the level of their metabolites. Due to its properties, moclobemide has a broad spectrum of antidepressant activity.

The selected metabolism test method assumes the use of human liver microsomes (HLM) with NADPH as a cofactor and thermal incubation. This method is cheap and fast, which is the reason of its frequent use in context of newly introduced drugs.

Ultra high performance liquid chromatography (UHPLC) coupled with accurate quadrupole-time-of-flight (Q-TOF) mass spectrometry was used to the metabolite profiling of moclobemide after the incubation with HLMs. The separation was performed on Kinetex C18 (dp=1.7 μm) column and gradient elution with a mixture of acetonitrile and 0.1% solution of formic acid in water was used. Mass spectrometry was performed in auto MS/MS mode in order to collect MS as well as all the fragmentation spectra of moclobemide and its metabolites. After two hours of *in vitro* incubation of moclobemide with HLMs six metabolites were found and structurally characterized. N-oxide derivative of moclobemide was identified to be the main metabolite of the analyzed drug. UHPLC coupled with Q-TOF high resolution MS method was found to be a powerful tool for the metabolite profiling of drugs after *in vitro* incubation with human liver microsomes.

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6.

Streamlined workflow to discover antibiotics

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Hyphenated planar chromatography (HPTLC-UV/Vis/FLD-EDA-HRMS) proved to be well-suited as a high-throughput bioanalytical tool [1] that can contribute in the discovery of new antibiotics. The *Bacillus subtilis* bioassay was directly applied in the chromatogram to demonstrate the streamlined strategy from screening, characterization and identification to bioquantification of natural antibiotics in root extracts of *Salvia miltiorrhiza* [2]. The sample preparation was kept simple to let the sample extract as native as possible. The bioassay applied in the chromatogram (bioautogram) eased the direct correlation of separated zones and effective zones. An inverse densitometric measurement was employed for bioquantification. The importance of two unknown antibiotics was specified via bioequivalency calculation. As a reference, cryptotanshinone was used. The overall antimicrobial result obtained was referred to the activity of two synthetic antibiotics, ciprofloxacin and marbofloxacin. These calculations were performed in a single run on the same plate. This strategy can be installed in every analytical laboratory without much microbiological effort. Any type of bacteria can be selected, depending on the effect of interest, and applied on the plate. Especially the linkage to microbiological assays with pathogenic bacteria will be of high relevance, in combination with HRMS/NMR/IR and bioquantification. A planar chromatographic approach for streamlined structure elucidation was recently reported [3]. The demonstrated potential of this bioprofiling can contribute to discovery of new antibiotics from natural sources.

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7.

Validation of RP HPLC method for quantitative analysis of antiretrovirals drugs in human plasma samples

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Therapeutic drug monitoring (TDM) of antiretrovirals requires accurate and precise analysis of plasma drug concentrations. This work describes a simple and sensitive UV HPLC method for determination of the commonly used protease inhibitors such as darunavir, lopinavir, ritonavir, fosamprenavir, tenofovir a nucleoside reverse transcriptase inhibitor (NRTI), the non-NRTI such as efavirenz, and *Dolutegravir* an integrase inhibitor . The diazepame internal standard was added to plasma aliquots prior to protein precipitation with methanol and acetonitrile. This method employed highperformance liquid chromatography with PDA detector. All compounds eluted within 30-min run time. Calibration curves were validated, with correlation coefficients (r) higher than 0.998, for analysis of therapeutic concentrations reported in the literature. Inter- and intra-assay variations were <15%. Evaluation of accuracy shows a deviation <15% from target concentration at each quality control level. No significant matrix effect was observed for any of the antiretroviral studied. This new validated method fulfills all criteria for TDM of antiretrovirals and was successfully applied in routine TDM of antiretrovirals.

8.

Application of a polynomial modified Gaussian model and a half-width plot approach to describe chromatographic peaks and reveal column performance in chaotropic chromatography

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Theoretical understanding and accurate prediction of analytes chromatographic behavior is very important for the development of an efficient separation method. We have proposed the three−step procedure of polynomial modified Gaussian model (PMG) and successfully used it to predict amlodipine and its impurity A retention behavior in chaotropic chromatography. In this study, we extended the applicability of three−step PMG procedure and tested its capability to relate the shifts in retention behavior of risperidone and its three impurities with the change of quantitative and qualitative parameters in chaotropic chromatographic systems. The experimental plan was defined by D−optimal experimental design due to its capability to assess both quantitative and qualitative parameters. The studied quantitative parameters were: acetonitrile content in the mobile phase (20 %, 30 %), the pH of the aqueous phase (3, 5) and the content of chaotropic agents in the aqueous phase (10 mM, 100 mM); while the qualitative parameters were: type of chaotropic agent ($NaClO_4$, NaTFA) and column type (Zorbax Extend, Zorbax Eclipse). Firstly, the appropriate models for retention factors of peak beginning (k_B), peak apex (k_A), peak ending (k_E), as well as for peak heights (H_0) were defined. Subsequently, indirect modeling of the following parameters: peak width at 10% of peak height ($W_{0.1}$), individual values of left half−width (A) and right half−width (B), number of theoretical plates (N) and tailing factor (Tf) was performed. Afterwards, the investigated experimental domain was divided by discretization to acquire a grid of appropriate density. On the basis of the predicted results for Tf and N and the defined criteria for the furthersimulation, the optimal region for solutes' separation was selected for every combination of qualitative parameters. The appropriate agreement between the predicted and experimental values verified the ability of three−step PMG procedure to successfully simulate the influence of quantitative and qualitative parameters on the solutes retention behavior in chaotropic chromatography.

Apart from unacceptably low retention, protonated form of basic analytes in reversed−phase HPLC (RP−HPLC) elute as highly deteriorated peaks due to secondary interactions with free silanol groups of the stationary phase. A half-with plots approach represents valuable and simple tool for predicting peak shape, but also column performance and its kinetics. Therein, this approach was used in the order to characterize performance of stationary phases with different end-capping in pH range close to pKa of silanol groups. The half−width plots were constructed using indirectly modeled values of left half−width (A) and right half−width (B). According to the peak broadening rate (r_{PB}), Zorbax Eclipse column, with tremethylsilyl end-capping, expressed better characteristics than Zorbax Extend column where bidentate bonding was used to block free silanol groups.

9.

Robustness testing of chaptropic chromatpgraphy method for the determination of olanzapin and its two impurities

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According to ICH Q2 robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal application. The aim of robustness testing is the identification of the factors with significant influence on the metod qualitative or quantitative performances. In this study, robustness testing of a chaotropic chromatographic method for the determination of olanzapine and its two impurities (impurity B and C) is performed. Plackett–Burman design was applied to discern the significant/influential factors. The factors to be investigated in a robustness testing are selected based on the conclusions from method development and optimization. Six quantitative factors (acetonitrile content in the mobile phase, sodium perchlorate concentration in water phase, pH of the water phase, column temperature, flow rate, detection wavelength), two qualitative factors (mode of mixing mobile phase constituents and stationary phase type), and three dummy factors were included in experimental plan. Peak areas of olanzapine and its impurities were selected as quantitative responses (*area O, area B* and *area C*).

The traditional approach of examining the results obtained by Plackett–Burman design assumed that the factor interactions do not affect the model significantly, so they can be neglected. If the model defined only with main factors has unsatisfactory statistical parameters, it is completely useless and a reevaluation of the model, including analysis of interactions contribution, should be carried out. In order to improve the accuracy and reliability of our initial models *demasking large dummy effects* (DDE) approach was used. In the first step the factor effect estimates were ranked and the main and dummy factors with the greatest effects were selected. Further on, the impact of every factor interaction was evaluated with the aid of alias matrix and used for the identification of important factor interactions. New models were defined comprising main factors with greatest effect and identified important interactions. These models showed significant improvement of statistical parameters (coefficient of determination, R^2 and adjusted coefficient of determination, *adj.* R^2) compared to initially proposed models with low and slightly low determination coefficient. The model improvement is achieved by adding three (*area B* and *area C*) and four interaction terms (*area O*) which makes model simple for real interpretation.

10.

Influence of selected mobile phase properties on the TLC retention behavior of ziprasidone and its impurities

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Properties of solvents used for chromatography significantly influence retention behavior and separation of the analytes. Selection of an appropriate mobile phase mixture is based mainly on interactions of solvents with the analytes and stationary phase. In this study influence of nine different solvents alone or in a mixture, a total of 19 mobile phases, on the retention behaviour of ziprasidone and its five impurities was examined by normal phase thin-layer chromatography in order to find the best mobile phase composition for separation of ziprasidone and its five impurities. Migration distances (MD) of the examined compounds obtained under the examined chromatographic conditions were correlated with calculated mobile phase properties, such as Snyder polarity and Hansen solubility. Both, linear and polynomial relationships were evaluated and equations with the best statistical parameters were selected. High correlation coefficients ($r > 0.706$) and satisfactory statistical parameters were obtained for mathematical relationships between the Snyder polarity or Hansen solubility parameters of mobile phases on the one hand and experimentally obtained migration distances of impurities I, II, V, and ziprasidone on the other. None of the mobile phase properties can be correlated with the retention behavior of impurities III and IV, but their retention behavior can be reliable predicted from the respective differences between the MD values, ΔMD(I–III) and ΔMD(IV-V). The obtained results indicate that for the selected polarity of mobile phase, migration distance of the impurity can be easily calculated.

11.

TLC-bioautography as an appropriate technique for screening anti-*haemophilus* activity of essential oils

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Essential oils (EOs) have been widely used for antimicrobial, medicinal and cosmetic purposes. EOs and their components are becoming increasingly popular as naturally occurring antimicrobial agents, however, the reliability of the common antimicrobial assays used for EOs is questionable. The aim of this study was the evaluation of antimicrobial properties of cinnamon (*Cinnamomum verum* J. Presl.), clove (*Syzygium aromaticum* (L.) Merr. and Perry), peppermint (*Mentha x piperita* L.), thyme (*Thymus vulgaris* L.) EOs and their main components against the Gram-negative bacteria, *Haemophilus influenzae* (DSM 4690) and *H. parainfluenzae* (DSM 8978).

The chemical composition of the EOs was measured with gas chromatography – mass spectrometry (GC-MS). Thin-layer chromatography – direct bioautography (TLC-DB) was used for the detection of the antibacterial activity of EOs, which is an appropriate method for investigation of complex extracts. EOs (100 µL) were diluted in 500 µL of absolute ethanol. From this solution, 0.5 µL and 1 µL were applied to the TLC plates (aluminium foil-backed silica gel TLC plates, Merck, Germany). An aqueous solution of MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyltetrazolium bromide (Sigma-Aldrich Ltd.) was used for the visualization of inhibition zones (expressed in the diameter, mm). Standards of eugenol, cinnamaldehyde, menthol and thymol (Sigma-Aldrich Ltd.) were also involved in TLC-DB experiment.

Both bacteria were the most sensitive to cinnamon EOs (19,5 mm) and cinnamaldehyde (23 mm). The EOs of thyme and clove were also effective with 11 mm and 12,5 mm diameters of inhibition zones, respectively. Peppermint oil showed weaker activity (8 mm). To the best of our knowledge, we performed TLC-DB with *Haemophilus* species firstly.

12.

Comparative analysis between chromatographically and computationally estimated lipophilicity descriptors of synthetic rhamnolipids

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Rhamnolipids are glycolipids of biological origin with an amphiphilic character. Mainly owing to their significant surface-active property, rhamnolipids have attracted much attention in recent years and, therefore, many new applications of these biomolecules have been suggested. Very intriguing therapeutic potential has been recognized in the light of the fact that rhamanolipds can inhibit biofilm formation. Bacterial biofilms are surface-adherent, multicellular communities which show increased antibiotic resistance and tolerance compared to free-living planktonic forms. Therefore, in the context of drug discovery which is, at target identification phase, consisted of high-throughput screening of numerous rationally proposed compounds, rhamnolipids manifestly represent good starting structures for design pathway of antibiofilm drugs. Aiming to deliberate mentioned concept, five rhaminolipids have been synthesized and characterized.

Since it has been noticed in the past that one of the main reasons for high attrition rates for compounds entering clinical trials is their poor pharmacokinetics and bioavailability, drug development in its early phases and parallel with structure−activity relationship (SAR) studies includes knowledge of absorption, distribution, metabolism, excretion, and toxicity (ADMET) of a drug candidate. Within current strategy, lipophilicity, log*P* has been distinguished as a key property that directs clinical success of drug candidate. The importance of this physico-chemical attribute is a result of its ability to determine solubility of a compound, compound's passive diffusion through biological membranes and the affinity for the target, as well as, how long compound will remain active in the body. Hence, determination of lipophilicity represents an imperative and noteworthy task in terms of prediction of the biological activity of investigated compounds.

From the analytical point of view, widely accepted reference system for measuring log*P* values is 1-octanol–water system. However, some significant disadvantages which have been noticed so far and which are connected to this traditional shake-flask method, implied strong need for finding simpler, yet more accurate experimental method for the determination of lipophilicity. As a consequence, measuring lipophilicity by means of chromatographic and computational techniques lately has gained popularity and significant base of new lipophilicity parameters has been generated.

The aim of the present study was to determine measures of lipophilicity using chromatographic techniques and to compare derived indices with computationally estimated lipophilicity scales. In order to find the most appropriate experimental method or computational approach for the determination of lipophilicity of proposed substances, comparative analysis was performed using novel ranking method based on the sum of ranking differences, SRD that had been developed by *Hébergerb K.*

Finally, based on the obtained results, a good perspective for extension of research in area of modeling biological activity of tested rhamnolipids (QSAR) regarding their lipophilicity is provided. Consideration of rhamnolipids of the most promising pharmacokinetic profile among the studied series, would be an ultimate goal related to further development of antibiofilm drug.

13.

Lipophilicity of some quinothiadiazine derivatives

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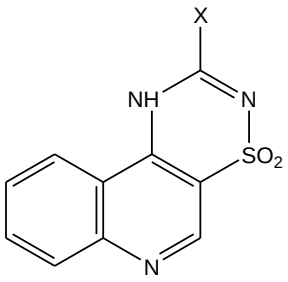
Compounds containing azine moiety are of interest due to their biological activity. Several of them are potential antitumor agents.

Lipophilicity is an important factor in determinating the biological activity of drugs. RP TLC is a very useful method for determination of lipophilicity. We used this method in our study of selected quinothiadiazines:



X = Me, OMe, $N(Me)_2$, NH(n-Bu)

and its N-methyl derivatives.

TLC was performed on silica gel RP-18 F_{254} plates (Merck #115389), activated by heating at 100 °C for 1 h. Mixtures of methanol-water solutions were used as mobile phases. Relationship between R_M values obtained from chromatography and partition coefficients is described by the equation $R_M = a \log P + b$, where a and b are constants for a particular system and P is the partition coefficient.

Coefficients a and b were found in experiments for compounds with known log P values. Log P values obtained for the investigated compounds were correlated with values calculated by several theoretical methods. Lipophilicity of investigated quinotiadiazines was compared with lipophilicity of antitumor agents: irinotecan and hesperidin.

14.

Determination of 1,2-dichloroethane as relevant impurity of ethephon using HS-GC-MS technique

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Plant protection products (PPPs) placed on the market should have sufficient quality, i.e. they should meet the technical requirements established for them in the process of their registration. It is especially important that the permitted concentrations of so-called relevant impurities are not exceeded. According to SANCO/3030/99 rev.4 document, relevant impurities are defined as "impurities of toxicological and/or ecotoxicological or environmental concern which are known, or can be expected, to occur in the active substance as manufactured."

Recent years a significant increase in the number of plant protection products marketed in Poland containing ethephon as the active substance was observed. According to the FAO specification, 1,2-dichloroethane is a relevant impurity of ethephon. The maximum permissible concentration of 1,2-dichloroethane in the preparation should not exceed 0.5 g/kg of ethephon, i.e. 0.05% The aim of this work was to develop a method for determination of the relevant impurity of ethephon in plant protection products in form water soluble concentrate (SL) using surface-to-surface analysis combined with mass spectrometry (HS-GC-MS).

15.

Office Chromatography - do it yourself!

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The Office Chromatography concept combines all relevant steps for online miniaturized planar chromatography by a single device [1, 2]. 3D printing of silica gel layers was recently demonstrated to be integrable into this concept [3]. This success outlined the potential of a 3D printing environment in planar chromatography and opened new avenues and new perspectives for tailor-made plates. Inspired by the do-it-yourself maker society, the 3D printing of thin silica gel layers was realized using open-source packages to encourage re-use and improvements and to stimulate the users to contribute to this emerging technology. All modifications of hard- and software for 3D-print of planar separation media were released open-source. A self-designed slurry doser replaced the plastic extruder of an open-source self-mounted Prusa i3 printer. Investment costs for the modified hardware were 630 Euro. After investigation of the optimal parameters for layer print, planar chromatographic separations were successfully demonstrated on these printed layers. Printing a 0.2-mm layer on a 10 × 10 cm format took less than 5 min, at running costs less than 0.25 Euro. Printed plane layers were compared with printed channeled layers. Therefore, 40 channels were printed on a 10 × 10 cm format for the separation of 40 samples in parallel, at running costs below 0.04 Euro. The printing process of such a channeled plate took only 2 min. New perspectives for tailor-made plates were opened with regard to layer materials, their combinations, gradient plates, different layer shapes and patterns. Streamlined open-source-based software for image evaluation of planar chromatograms, termed rTLC, was recently developed [4]. The integration of printing of sample solutions and mobile phase is in progress. Its combination with mass spectrometry (MS) [5, 6] and bioassays [7-10] in the near future will proof its potential for high-throughput microscale effect-directed analyses (EDA). Office Chromatography might be the next important tool in the analytical toolbox of experts!

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