

POSTER SESSION II THURSDAY, JUNE 9th, 2011

CHAIRPERSONS: D. Mangelings and Ł. Komsta

15.

Photostability Study of Alopres® tablets

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Amlodipine besylate is a long-acting calcium channel blocker (dihydropyridine class), that is in use for the treatment of angina [1] and hypertension [2]. Today can be found many products with amlodipin or its combination with other substances on the market. In the study were investigated the Alopres® tablets (Zdravlje-Actavis, Serbia), which contain only amlodipine besylate as an active substance. Since the amlodipine besylate is photosensitive compound [3,4] the main aim of this study was to examine the photostability of the Alopres® tablets (Zdravlje-Actavis, Serbia). Also, the photostability of the Alopres® tablets was compared with related commercially available products. All the examined tablets were stored under accelerated photo conditions, in accordance with ICH regulative [5]. The content of the amlodipine besylate in the irradiated samples was determined by use of validated RP-HPLC method.

Key words: Alopres, amlodipine, tablet, photostability.

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16.

Solid phase extraction for the determination of biogenic amines in dry fermented sausages

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During the fermentation of dry sausages the amount of biogenic amines (BAs) increases due to the microbial decarboxylation of amino acids. The monitoring of the BAs is therefore recommended in the course of biogenic amine intoxication. The BAs were separated and detected by means of C18-HPLC-UV after derivatization with dabsyl-chloride. Prior to analysis, the target compounds were extracted from the meat samples with 0.4 M HClO₄. In complex protein-fat matrices, an extra clean-up step can improve the baseline separation of the target analytes.

In this study the feasibility of a C18-SPE as purification step was explored. An experiment was set up to determine the best possible conditions for the SPE procedure. Therefore the different eluted fractions were collected separately and each one was analyzed for the target compounds. Initially, SPE purification was directly applied after the perchloric acid extraction. After activation of the cartridge with subsequently water and acetonitrile, the sample was loaded and washed with water. Elution of the BAs was forced with acetonitrile. Under these conditions, the BAs were distributed over the different fractions, i.e. loading, washing and elution, due to the great polarity differences of the underivatized BAs. Therefore, the extract was, prior to SPE, derivatized with dabsyl chloride. In that way, the BAs become more apolar and were better retained in the course of washing. But even then, still a small percentage of BAs, mainly phenethylamine, eluted during loading and washing. To ensure the retention of all BAs during washing, 0.4 M HClO₄ was used instead of water.

The final SPE procedure existed of following steps: activation with 0.4 M HClO₄ and acetonitrile, loading of the primarily dabsylated extract, two washing steps with perchloric acid and two elution steps with acetonitrile. Introduction of this modified SPE procedure improved the sensitivity of the method by the possibility to concentrate the sample in an absence of interfering compounds.

17.

Determination of the soil-water, octanol-water, and air-water partition coefficients for the twelve benzodiazepines by the means of the reversed-phase thin-layer chromatography

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The omnipresent and raising concern about environmental fate of pharmaceuticals has been the main driving force in developing and establishing novel methods for rapid and inexpensive estimation of many of the ecologically important parameters such as the soil-water or air-water partitioning constants. In the present study reversed-phase thin-layer chromatography using octadecyl-modified silica as a stationary phase and dioxane-water or acetonitrile-water mixtures as mobile phases was employed. Mathematical models relating retention parameters of several standard compounds with experimentally obtained partition coefficients were established, with satisfactory statistical properties. Calibration models were further used to predict – determine the soil-water ($\log K_{OC}$), octanol-water ($\log K_{OW}$), and air-water ($\log K_{AW}$) partition constants of twelve benzodiazepine compounds mostly used on Serbian market. The obtained values were further compared with the calculated ones by the several computational methods mostly employed through the EPI suite software, which is freely available from the US Environmental Protection Agency (EPA). Good accordance was established among several parameters, which indicate that reversed-phase thin-layer chromatography could be used as a relatively cheap and fast method in assessment of the aforementioned parameters.

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18.

Degradation of C.I. Reactive Black 5 using water falling film dielectric barrier discharge. An investigation of carboxylic intermediates by IC

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Textile manufacturing is one of the largest industrial producers of wastewater, which have high concentrations of organic and inorganic compounds and strong color, caused by residual dyes that were not fixed to the fibers in the dyeing process. Azo dyes were chosen due to the fact that they are the major colorants in the textile industry; they provide colors with outstanding colorfastness and wide spectrum. At the same time, azo dyes are the most toxic, mutagenic and carcinogenic commercial dyes [1].

In the present paper, the degradation of commercial reactive azo dye C.I. Reactive Black 5 was studied using Advanced Oxidation Processes (AOPs) in a non-thermal plasma reactor, based on coaxial water falling film Dielectric Barrier Discharge (DBD) [2]. Degradation products, which resulted from the oxidative degradation process, were determined by the use of ion chromatography. The effectiveness of subsequent recirculation of dye solution through the DBD i.e. dye conversion into carboxylic intermediate was monitored. Initial dye concentrations in the solution were 40, 80, 200, 500 and 1000 mg/L. Anions of interest were separated on Dionex AS11 column with conductivity detector, with potassium hydroxide as mobile phase. Due to low retention of acetate and formiate and high retention of oxalate and fumarate on aforementioned column gradient elution was applied.

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Revisiting thin layer chromatography as a lipophilicity determination tool.

Part II. Is silica gel a reliable adsorbent for lipophilicity investigation ?

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As many approaches regarding lipophilicity determination with TLC (different modifiers and treatment of retention data) are mixed together and almost randomly chosen in every-day published studies, the subject needs some contribution of standardization. The problem is very comprehensively discussed in the case of HPLC, comparative studies by TLC were almost undone.

As the recent articles often present the use of silica gel layers (normal phase) in lipophilicity estimation, the purpose of the second part of our study¹ was to compare several approaches of TLC lipophilicity determination on this adsorbent: a single TLC run, extrapolation of a retention, principal component analysis of a retention matrix, PARAFAC on a three-way array and a PLS regression.

All techniques were applied to 35 model solutes with simple molecules, using silica gel 60 F254 thin layer plates and nine concentrations of six modifiers: acetone, dioxane, ethyl acetate, methylethylketone, propan-2-ol and tetrahydrofuran.

Comparative analysis formed several general recommendations, similar to previous part on RP18 plates:

1. Propan-2-ol and tetrahydrofuran were the best modifiers, while acetone gave the worst correlation of retention with lipophilicity.
2. Surprisingly good correlations were obtained for single TLC runs and this method is underestimated in the literature.
3. Advanced chemometric processing proposed recently, such as PCA, PARAFAC and PLS did not show a visible advantage comparing to classical methods.

Although the retention mechanism on silica is not connected with partitioning between two phases, there is a significant correlation between experimental lipophilicity and retention in silica gel. This adsorbent can be used to estimate lipophilicity, but the correlation is a little bit worse than on reversed-phase systems.

¹ The first part was presented in Szczyrk in 2010 and then published in *J. Pharm. Biomed. Anal.* 2010, 53, 911-918

20.

Densitometric RP TLC-DPPH method for quantitative evaluation of free radical scavenging activity of N,N'-bis(acetylaceton)ethylenediimine and corresponding copper(II) complex

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Schiff bases and their complexes with metals have provoked wide interest in their diverse spectra of biological and pharmaceutical activities, such as anticancer, antitumor and antioxidative due to structural similarities with natural biological systems. Besides, the Schiff base complexes can be greatly modified by introducing different substituents. They provide models for different chemical processes and investigations. Free radicals are species that contain unpaired electrons. Therefore, they may induce some oxidative damage to biomolecules thus accelerating ageing, cancer, cardiovascular diseases, neurodegenerative diseases and inflammation. There is a parallel increase in the use of methods for estimating the efficiency of such substances as antioxidants. One such method that is currently popular is based upon the use of the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH).

The aim of this work was development of method which gives the possibility of determination of antioxidative activity of compounds independent of applied solvent. N,N'-bis(acetylaceton) ethylenediimine and corresponding copper(II) complex were used for this investigation. Antioxidant activity was determined in vitro and has been evaluated using a RP TLC method that involves reaction between DPPH (2,2-diphenyl-1-picrylhydrazyl) and Schiff base and its metal complex. Investigated compounds were applied by autosampler followed by application of methanolic solution of DPPH on the same spots. The antioxidant activities, expressed in Trolox Equivalent Antioxidant Capacity (TEAC), were determined on the basis of a calibration plot, peak height being a function of Trolox concentration. Their antioxidant capacities were quantitatively evaluated using densitometry with detection at 515 nm. The strength of antioxidant activity of Schiff base metal complex was shown to be significantly stronger than antioxidant activity of its ligand.

21.

Application of gas chromatography in experiments on steam gasification and co-gasification of coal and biomass

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Sustainable energy management requires utilization of renewable energy resources to the possibly widest extent, especially in the light of continuous increase in energy demand and recognition of environmental problems related to fossil fuel processing. In Poland, with the energy sector traditionally based on coal, this issue has also gained a considerable recognition reflected in targets related to diversification of energy resources set in the energy policy until 2030. An increasing trend in the renewable resources share in the final energy use (to about 15% in 2020) as well as support given to the development of distributed energy systems and highly efficient technologies, like gasification is expected.

In the paper the results of experimental comparative study on steam gasification of lignite, hard coal and energy crop – derived biomass (*Salix Viminalis*) in a laboratory-scale fixed bed reactor at the temperature of 700°C were presented. The amount and composition of product gas were measured and analyzed online via flow meter and gas chromatograph Agilent 3000A, respectively. The main component of synthesis gas produced in the process of steam gasification was hydrogen. The highest hydrogen content in product gas was observed for lignite samples (66-67%vol.). The respective values for hard coal and biomass samples were comparable (59-64%vol. and 59-62%vol., respectively). The experiments on fuel reactivity in the process of steam gasification proved the biomass to be highly reactive in the process of steam gasification. However, the calorific values of product gas in biomass gasification tests were relatively lower than the respective values for lignite and hard coal. The product gas yields in coal gasification tests were over twice the volumes of the biomass-based gas. These results constituted the basis for further experiments focused on co-gasification of coal and *Salix Viminalis* blends of biomass mass fraction of 20, 40, 60 and 80wt%. Hydrogen content in product gas increased with an increase in process temperature for all biomass/coal ratios. A synergy effect at all tested temperatures was observed in co-gasification tests for blends with 20 and 40wt% content of biomass, consisting in an increase in the volume of product gas, when compared to the tests of coal and biomass gasification. In tests of co-gasification of blends of higher biomass mass fraction (i.e. 60 and 80wt%) the opposite trend was observed.

Application of gas chromatography in experiments of underground lignite gasification to hydrogen-rich gas

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The efforts of the world research society focus on the development of new, environmentally friendly, economically attractive and commonly accessible energy carriers. Hydrogen is considered as the clean alternative fuel, the production of which may be based on the process of fossil fuels' gasification. In-situ coal processing might be an attractive option of hydrogen-rich gas production. In the process, the gasifying medium such as air, oxygen and/or steam is injected into a coal seam through a surface well or from the post mining space on the level of coal deposits.

In the paper the results of the experimental study on simulated in-situ lignite gasification to hydrogen-rich gas with oxygen and steam are presented. The experiments were conducted in an ex-situ reactor, in which real underground conditions can be simulated both in respect to the coal seam and the surrounding rocks strata. The reactor was designed to carry out gasification experiments on extra large coal samples, so that to preserve the natural structure of the coal under study. The composition of the outlet gas mixture was analyzed online via gas chromatograph Agilent 3000A, whereas the amount of the gas produced in the gasification process was measured with a flow meter. The experiment was initially divided into three stages: the ignition stage, the oxygen stage and the steam stage. Gas produced in the steam gasification stage was characterized by the calorific value of 7.8 MJ/m^3 and average hydrogen content of 46.3% vol. A rapid decrease in the temperature levels and in the amount of produced gas proved that the tested lignite of 53% vol. moisture content was not suitable for steam gasification. A great amount of thermal energy was consumed for water evaporation which led to a considerable heat loss. An addition of stoichiometric amount of water in the system by adding steam caused the seam to extinguish. Thus only oxygen could be used as the gasifying medium in the gasification of the tested lignite. The average calorific value of gas produced during stable operation in oxygen gasification stage equaled 5.2 MJ/m^3 with the average gas production rate of $16.0 \text{ m}^3/\text{h}$ and the average hydrogen content in the produced gas of 26.4% vol.

The HPLC analysis of the selected extract fractions derived from a variety of the sage (*Salvia*) species

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The sage (*Salvia*) genus embraces over 900 different sage species, which are widespread all over the globe [1]. Many of these species are used in natural medicines of different cultures, basically due to a wide spectrum of their biological activity (like, e.g., antioxidative, anti-tumor, and antibacterial properties). With time, classification of the different sage species has become increasingly more difficult, as in terms of morphology, many of them look quite similar. Moreover, many different sage species appear in form of the subspecies, holding their individual botanical names [2].

Recently, a lot of attention has been paid to biologically active and water-soluble compounds contained in sage. These are basically polyphenol flavonoids, phenolic acids, and simple coumarins. The majority of phenolic acids contained in the different sage species are caffeic acid derivatives. Caffeic acid depsides (i.e., rosmarinic and chlorogenic acid) are considered as the main biologically active compounds contained in sage [1]. According to the literature, rosmarinic acid is the main compound responsible for antioxidative properties of the sage genus [3]. The analysis of chemical composition of the sage extracts most probably can help to better understand the biological potential of this genus and the systematic relations among the individual sage species.

At the first stage of this study, we focused our attention on spectrophotometric determination of the sums of phenolic acids and flavonoids in the sage extracts with use of the pharmacopeial procedures [4]. To this effect, two separate extractions were carried out, with use of the Arnov's reagent (phenolic acids) and aluminium chloride (flavonoids). These preliminary measurements allowed to select nine out of twenty six sage species with the highest contents of phenolic compounds (which were *S. amplexicaulis*, *S. azurea*, *S. cadmica*, *S. glutinosa*, *S. pratensis*, *S. pratensis ssp. Haematodes*, *S. sclarea*, *S. staminea* oraz *S. triloba*). At the second stage of this study, the nine selected sage species underwent an exhaustive liquid – solid extraction in the Soxhlet apparatus with the liquid – liquid purification step. Finally, the qualitative and quantitative assessment was performed with use of high-performance liquid chromatography (HPLC) of individual fractions containing flavonoid glycosides and aglycones, free phenolic acids and flavonoids, and also phenolic acids and flavonoids liberated through the acidic and basic hydrolysis. This study makes part of a wider research project targeting chemical composition of a variety of the sage species.

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Thin layer chromatography with micellar mobile phase of aromatic biogenic amines in magnetic field

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Constant need for solving various new analytical problems results in research on new methods and techniques. In some cases, it is enough to modify an old technique to overcome the difficulties and receive a satisfying solution.

Using micellar mobile phase in liquid chromatography is one of those cases. By adding a surfactant in concentration higher than CMC, chemical properties of mobile and stationary phases (and in consequence the whole chromatographic system) change drastically, and open a totally new area in chromatographic analysis. Although the exact mechanism of retention in micellar liquid chromatography is still unrecognised, it helps to solve many problems where other methods failed to come researchers' expectations.

Biogenic amines are a group of biologically active compounds responsible for very important processes in living organisms such as protein synthesis, DNA replication, metabolism and the growth of cells. They are also transmitters. Their conventional chromatographic separation, quantitative and qualitative analysis is very sophisticated. That is why, applying the external magnetic field, as an additional factor modifying chromatographic system, has been taken under consideration in this project.

The use of thin layer chromatography in our experiment allowed to compose and examine far greater number of chromatographic systems than it could be possible with any other chromatographic method at the same time. It made possible to lower organic solvent usage reducing environmental and financial costs of the whole project. It also simplified applying the external magnetic field to the experiment thanks to small dimensions of chromatographic chambers and plates.

In our survey, we decided to focus on three main subjects and ways of data analysis, concerning:

- optimisation and efficiency of separation of investigated amines in micellar chromatography – through the analysis of chromatographic data obtained for different kinds and concentrations of surfactants, organic modifiers, for various pH values and stationary phases ;
- influence of magnetic field on chromatographic systems with micellar mobile phase – by comparing results of chromatograms developments carried out in magnetic field and outside it.
- gathering additional information on biological behaviour of aromatic biogenic amines and their role in living organisms under varying chemical (pH changes) and physical (presence of external magnetic field) conditions – by the analysis of the whole data obtained during the experiment.

It is obvious that it is impossible to find all the answers in one project, but we hope that we will get closer to final solutions of problems presented above, and our work will be a small contribution useful in future discoveries.

Comparison of GC/MS, GC/ECD and HPLC/DAD techniques in determination of PBDEs in water samples

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The reason for undertaking this research was the Water Framework Directive, which defines framework of cooperation in the field of water policy. The directive gives parameters of water chemical state assessment made by indicating substances with proven or highly probable, especially harmful effect on ecosystems and water of so called priority substances.

The aim of this work was to devise chromatographic methods of determination Penta-BDE, Octa-BDE and Deca-BDE in water and waste water samples. For determination of analyzed substances, researchers used a gas chromatograph compound with mass spectrometer (GC/MS), high pressure liquid chromatograph with diode array detector (HPLC-DAD) and gas chromatograph with electron capture detector (GC-ECD). The following two methods of samples extraction were compared: the liquid-liquid extraction and solid phase extraction using different extraction solvents and absorption phases.

The best devised method with lowest limit of quantification was GC-ECD, which allows determination of Penta-BDE in water samples according to ordinance of the Ministry of Environment 20 August 2008 regarding the way of classification of surface water deposits state (DZ. U. Nr 162, poz. 1008).

**DEHYDRATION AND ANALYSIS OF 18-CROWN-6 USED FOR ANIONIC
POLYMERIZATION OF PROPYLENE OXIDE**

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Keywords: gas chromatography, size exclusion chromatography, anionic polymerization, crown ether.

18-Crown-6 made by four different companies with the declared purity on the level about 99.8 percent (Tab.1, Fig. 1) was used as an activator in the anionic polymerization of polypropylene oxide by oligo(potassium glycidol) as the macroinitiator. Two series of polymerization were conducted, i.e. before and after crown ether dehydration. The influence of 18-crown-6 purity was observed on the rate of polymerization and on the molecular mass of polymers obtained.

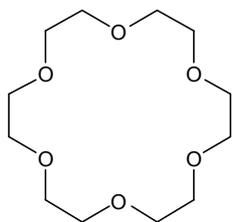


Fig. 1. 18-crown-6

1,4,7,10,13,16-hexaoxacyclooctadecane

Chemical Formula: C₁₂H₂₄O₆

Exact Mass: 264,16

m/z: 264,16 (100,0%), 265,16 (13,5%), 266,16 (2,0%)

Elemental Analysis: C 54.53; H 9.15; O 36.32

Tab. 1.

L.p.	Declared purity	Measured purity before dehydration	IR test
1	GC≥98,5%	GC/MS≥99,08%	passed
2	GC≥98%	GC/MS≥97,46%	passed
3	GC≈99%	GC/MS≥99,06%	untested
4	GC≥98%	GC/MS≥97,40%	passed

It was found that the SEC technique allowed to control the crown ether and poly(propylene oxide) purity. The GC-MS technique was successfully applied for qualitative and quantitative identification of impurities.

27.

New CZE-DAD and LC-DAD methods for honeybee venom analysis and standardization of the product

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Honeybee venom possesses diverse biological and pharmacological properties. It has been demonstrated its effectiveness in treating pathological conditions such as arthritis, rheumatism, pain, cancerous tumors and skin diseases. However, bee venom is a toxin and in order to develop pharmaceutical formulations for the safe administration, comprehensive information about its toxicology, side effects and chemical composition is needed. Moreover as it is natural product of high complexity uniform guidelines for standardization of this product are required.

The aim of this study was to develop and compare the new LC and CZE methods for honeybee venom characterization. The developed methods have been applied for analysis of bee venom samples of different strains of the bees, country of origin, year and season of the venom collection. At least nine honeybee venom constituents were separated and the content of four of them (apamine, mast cell degranulating peptide, phospholipase A₂ and melittin) have been determined. Applying LC and CZE the differences in chemical composition of honeybee venom were evaluated. Probably this was the first study in which the internal standard in chromatographic and electrophoretic assays of honeybee venom has been used.

The following steps and parameters were took into account for the validation of the method: selectivity, precision (injection repeatability, analysis repeatability), accuracy (recovery), linearity and operating range, limit of detection (LOD) and limit of quantitation (LOQ). All steps of validation proved that the developed analytical procedures were suitable for their intended purpose (standardization). It was stated that CZE and LC data did not differ significantly. Developed methods due to their simplicity can be easily automated and incorporated into routine operations both in the bee venom identification, quality control and assay tests. Moreover CZE was found to be a cheaper method than LC because of lower consuming of reagents used.

28.

Using gas chromatography to control the functioning of polytrioxane production installation

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The paper presents possible uses of gas chromatography method in the analytic control of the functioning of polytrioxane production installation. The polytrioxane belongs to the group of polyacetals which due to their proprieties are widely used by different branches of industry.

The basic material to produce polytrioxane is trioxane. In the Polish technology the method of trioxane production is based on trimeryzation of aqueous formaldehyde solution in the presence of sulphuric acid as a catalyst. For this purpose formaldehyde solution of ca. 60% is used. The chemical reaction takes place at the boiling point of the reaction mixture. The process of obtaining trioxane is not entirely selective. During the synthesis, different by-products of reaction are obtained in the reactor such as: methanol, formic acid, methyl formate and methylal. As the concentration of trioxane for polymerization should be at least 99,50%, it has to be further concentrated and purified through distillation, extraction and crystallization.

For the production of polytrioxane to be accurate, it is necessary to carry out regular analytic control of individual production stages and final product quality. Gas chromatography method, which offers a possibility of analyzing samples of complex organic mixtures, can be used in the ongoing control of trioxane synthesis.

29.

Application of GC analysis in the control of trioxane synthesis using ion-exchange resins as catalysts

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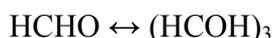
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The application of the GC method turns out to be useful in selecting a catalyst for the trioxane synthesis process. The method makes it possible to quickly identify and determine the concentration of reaction products and consequently the relevance of a catalyst.

Trioxane is a cyclic trimer of formaldehyde, a major monomer used in the polymerization to obtain polytrioxane. Polytrioxane is thermoplastic with very good mechanical properties, widely used in automotive, electronic and mechanical industries.

The production of trioxane consists in the trimerization of aqueous formaldehyde solution in the presence of acidic catalysts. The trioxane synthesis reaction is conducted at the boiling point of 103 – 110 °C.

The chemical equation is following:



Sulfuric acid is commonly used as a catalyst in this reaction. The use of sulfuric acid as a catalyst causes the following problems:

- low yield of the reaction,
- low selectivity of the process (large quantity of by-products such as: methanol, formic acid, methyl formate, methylal),
- corrosion of installations,
- precipitation of paraformaldehyde.

Experiments were conducted to replace sulfuric acid with a different catalyst, witch would ensure a higher yield and selectivity of the reaction. Three acidic ion-exchange resins produced by Purolite were chosen for the experiment. The method of gas chromatography was used to determine the quality and quantity of the reaction products resulting from trioxane synthesis process. The GC method was used in order to determine the yield and selectivity of the reaction.

Determination of volatile components and phenolic acids in *Satureja montana* by GC/MS

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Our work presents the results of chromatographic analysis of the volatile fraction and the phenolic acids fraction contained in different samples of the culinary plant winter savory (*Satureja montana*). This herb is highly appreciated and widely utilized as a food additive and a medicinal plant (which improves functioning of human digestive system).

The first part of this study focused on the volatiles (i.e., on essential oils) contained in *Satureja montana*, which have been isolated by means of hydrodistillation. Two types of the hydrodistillation apparatus were used:

- the Clevenger apparatus (recommended by European Pharmacopoeia), and
- the Deryng apparatus (recommended by Polish Pharmacopoeia).

The aim of our research was to find out which system provides a better option for derivation of essential oils. The obtained results were additionally compared with those obtained, when using the headspace-GC/MS system as a reference technique in the analysis of the volatile fraction. The headspace analysis was carried out at 100°C (to imitate the temperature regime utilized in hydrodistillation). Chemical composition of essential oils was in each case determined by means of the GC/MS technique. The major components of the volatile fraction contained in winter savory proved to be thymol, carvacrol, α -terpinene, myrcene, α -thujene, and linalool.

The second part of our study was focused on the analysis of phenolic acids. To isolate these compounds from plant material, we used the techniques of solid phase extraction (SPE) and accelerated solvent extraction (ASE) of the residual plant material from hydrodistillation. Within the framework of the SPE technique, we carried out the acidic and basic hydrolysis of the samples first (to split the glycosidic and ester bonds, and to liberate free phenolic acids). Then the extracts were percolated through the octadecyl and quaternary amine SPE columns. The eluted samples were evaporated to dryness with an air stream and derivatized with the BSTFA+TMCS (99:1) mixture in pyridine. The parallel ASE extraction of phenolic acids was carried out from the plant material by means of petroleum ether. The phenolic acids containing extracts derived from *Satureja montana* by means of the SPE and ASE technique were analyzed with use of the GC/MS analytical system.

Stability study of acetylsalicylic acid in solutions by the use of UHPLC/ESI-Q-TOF method

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Acetylsalicylic acid also known as a aspirin is a very popular analgesic and antipyretic agent with a very labile properties to reveal its degradation to salicylic acid. However the stability of aspirin is well known, the literature data concerning its stability in organic solutions is very poor.

Ultra high performance liquid chromatography (UHPLC) coupled with accurate quadrupole-time-of-flight (Q-TOF) mass spectrometry was used to the stability study of acetylsalicylic acid in different solutions: methanol, ethanol, propanol, butanol, acetonitrile, tetrahydrofuran, dioxane and water. The separation was performed on Zorbax Extend-C18 (2.1x50mm, dp=1.8 μ m) HT column and mixture of acetonitrile (A) and 0.1% solution of formic acid in water (B) was used as a mobile phase.

The gradient elution was carried out at constant follow 0.4 ml/min from 15%A (85%B) to 80%A (20%B) 0 - 13 min and than 15%A (isocratic) 13-14 min. Assay was monitored by the use of DAD (190-400 nm) and Q-TOF (negative ionization) detection. Mass spectrometry was performed in auto MS/MS mode in mass range: 50-1100 m/z and with acquisition rate: 1.41 spectra/s. In this mode during one run full MS/MS spectra and also MS (TOF) spectra were recorded. Extracted ion chromatograms (EIC) from MS spectra for acetylsalicylic acid (179.03490 m/z) and salicylic acid (137.02440 m/z) were used for quantitative analysis of aspirin and its main degradation product in chosen solutions. Calibration range was 0.4 – 14 μ g/ml for acetylsalicylic acid and 0.02 – 8 μ g/ml for salicylic acid. Correlation coefficients were >0.999 in both cases.

Stability of acetylsalicylic acid in eight solutions at concentration 10 μ g/ml was tested in 24h period. Every 2 hour full MS and MS/MS spectra were collected and qualitative and quantitative analysis was performed. In all tested solutions only one degradation product – salicylic acid was found, however significant differences in the rate time of degradation were observed. The most stable solutions were: acetonitrile and dioxane above 80% of initial concentration of aspirin was found in this case (after 24h). The most fast degradation of analyzed compound was observed in butanol (after 10 hour aspirin was totally degraded) and in methanol and ethanol solutions (below 30% of initial concentration of aspirin was found after 24h). Interesting results were observed in the case of water solution (concerning 1% of methanol from stock solution), only about 30% of acetylsalicylic acid was resolved to salicylic acid after 24h.