POSTER SESSION II WEDNESDAY, MAY 26 th, 2010

CHAIRPERSONS: D. Mangelings and L. Vanden Bossche

1.

Role of biogenic amines in the formation of *n*-nitrosamines during meat processing

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The formation of *N*-nitrosamines in a wide range of foods, especially in meat and meat products, can be bait by different factors like the presence of sodium nitrite, conditions of heating, storage time, bacteriological status or pH. Their formation in the presence of biogenic amines (BA) creates an additional toxicological risk. Raw meat contains certain amounts of biogenic amines e.g., spermidine, spermine, cadaverine and putrescine. The concentration of the latest ones can increase due to bacterial proliferation under inappropriate storage conditions. Presence of biogenic amines in meat is monitored for quality reasons, because BAs can be used as chemical indicators for bacterial spoilage of the final products [1-3].

The aim of this study was to determine the role of biogenic amines i.e., putrescine, cadaverine, spermidine or spermine on the formation of *N*-nitrosamines in cured meat products. Such products were processed with different amount of sodium nitrites (0 mg kg^{-1}, 120 mg kg^{-1}, 480 mg kg^{-1}), 0 or 1000 mg kg^{-1} of the stated biogenic amines, and heated at 85°C, 120°C, 160°C and 220°C. Experimental evidence was produced using gas chromatography in combination with Thermal Energy Analyzer (GC-TEA). The obtained analytical results were statistically evaluated by means of the Univariate Analysis of Variance (ANOVA) approach.

From the obtained data could be concluded that higher processing temperatures and higher added amounts of sodium nitrite increase the yields of *N*-nitrosodimethylamine (NDMA). Addition of cadeverine and spermidine caused a significant increase on the *N*-nitrosopiperidine (NPIP). The other added amines, i.e. putrescine and spermine, did not had a measurable influence on the nitrosamines concentrations. Beside *N*-nitrosopyrrolidine (NPYR) in some rare cases, no other volatile *N*-nitrosamines are detected.

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2.

Development of the method for determination of ziprasidone and its impurities

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Ziprasidone is an atypical antipsychotic drug, belongs to second generation, which possesses high affinity for adrenergic (α_1), histamine (H_1), and serotonin (5-HT_2) receptors as well as dopamine (D_2) receptors. It is used for the treatment of schizophrenia and in acute manic or mixed episodes associated with bipolar disorder. For acute agitation in patients with schizophrenia, ziprasidone may be given as the mesilate by intramuscular injection.

As it is not official active pharmaceutical ingredient in European Pharmacopoeia, there are not so many data available on simultaneous quantification of ziprazidone and its impurities in bulk powder and in dosage forms. Therefore, the purpose of this investigation was to develop and validate a selective RP-HPLC method for analysis of ziprasidone and its five impurities which differ in polarity and pKa_s. During the preliminary study some important data about chromatographic behavior of substances were found. Relationship between chemical structure and chromatographic elution was established.
Satisfactory chromatographic separation - good resolution, peak symmetry, retention and selectivity factor, was achieved using combination of gradient and isocratic elution. The most important factors in the separation were column type and the profile of chromatographic elution. Separation were performed on the Hewlett Packard 1100 Series chromatographic system (Agilent Technologies, Germany) with column Waters Spherisorb® ODS 1, (4.6mm×250 mm, 5.0 μm). Injection volume was 40 μl. All analyses were performed at 25 ◦C and the UV detection was performed at 250 nm using diode array detector. Mobile phase consists of water phase - 1% TEA in 0.05M potassium dihydrogen phosphate solution whose pH was adjusted to 2.5 by orthophosphoric acid, and organic phase – acetonitrile. The flow rate was 1.5 ml/min and run time was 20 minutes.
Relationship between peak areas and the amounts of the active pharmaceutical ingredient and all of five impurities was proven by validation parameters - linearity, recovery, precision and quantitation limit, which found to be satisfactory. Finally, method is convenient for the purity control of ziprazidone and assay, both in raw materials and dosage forms.

3.

Spirocyclopropane-type Sesquiterpene hydrocarbons from *Schinus terebinthifolius* RADDI

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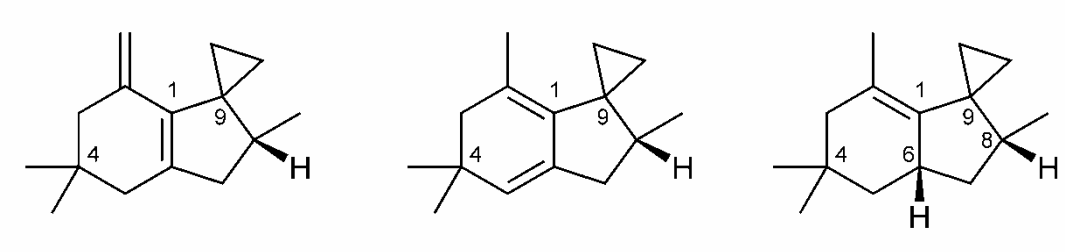
Schinus terebinthifolius RADDI is an evergreen shrub or tree of the Anacardiaceae, native to South and Central America. The leaves and reddish fruits are rich in essential oil. Earlier investigations reported high concentration of monoterpenes (Stahl, 1983; Malik, 1994) along with some sesquiterpene hydrocarbons (Singh et al., 1998).
In order to obtain additional data, dried fruits of S. *terebinthifolius* were hydrodistilled (Sprecher, 1963) to afford the essential oil, which was investigated by GC and GC-MS (GC-column: CP-Sil-5). A combination of column chromatography on silicagel 60, preparative GC on polysiloxane SE-52, and repeated semipreparative GC using megabore thickfilm capillary columns coated with DB-1 and DB-1701, three unknown sesquiterpene hydrocarbons (1, 2, 3) were selected. Structure assignments were carried out by using NMR spectroscopy*.

1 **2** **3**



These new natural products are 9-spiro(cyclopropa)-4,4,8-trimethyl-2-methylenbicyclo[4.3]non-1(6)-ene (terebanene, **1**), 9-spiro(cyclopropa)-2,4,4,8-tetramethylbicyclo[4.3]nona-1,5-diene (teredenene, **2**), and (6*R**,8*R**)-9-spiro(cyclopropa)-2,4,4,8-tetramethylbicyclo[4.3]non-1-ene (terebinthene, **3**).

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* In press

4.

Spectrophotometric determination of the sum of phenolic acids and flavonoids contained in twenty different sage (*Salvia* L.) species and the analysis of the sage extracts by means of HPLC-DAD and HPLC-ELSD

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Phenolic acids and flavonoids are the two groups of chemical compounds widespread in the plant kingdom that exert a positive influence on human health. Their particularly important biological role consists in an antioxidative and radical-scavenging activity. The group of phenolic acids embraces the highest number of active compounds which play a significant role in various different defensive mechanisms in the plant organisms (e.g., protecting them against infections, an excessive exposure to sun, or injuries [1]). The main biological role of flavonoids in humans relates to the blood vessels and to the blood circulatory system, although certain flavonoids exert even a more complex impact on human health.

The objective of this study was spectrophotometric determination of the sum of phenolic acids and flavonoids contained in the extracts originating from twenty different sage (*Salvia* L.) species, and the chromatographic fingerprint analysis of the respective extracts by means of high-performance liquid chromatography with the diode array and the evaporative light scattering detectors (HPLC-DAD and HPLC-ELSD).

Botanical material originated from the Pharmacognosy Garden of Medical University, Lublin, Poland, harvested in the three consecutive vegetation seasons (i.e., in 2007, 2008, and 2009). This material has been extracted in the two different ways proposed in the literature [2], one targeting phenolic acids and the other flavonoids.

The main aim of spectrophotometric and chromatographic analysis was to expose seasonal and inter-species composition differences in the contents of phenolic compounds. By means of the HPLC analysis, the DAD and ELSD fingerprints of the scrutinized extracts were collected. Moreover, the chromatographic analysis served an identification purpose also, and it was carried out against the acquired phytochemical standards.

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[2] Farmakopea Polska VII, Polskie Towarzystwo Farmaceutyczne, Warszawa 2006

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5.

Chromatographic and spectroscopic analysis of essential oils from *Salvia lavandulifolia* L. and *Salvia triloba* L.

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Mixtures of volatile compounds which can have a diverse chemical nature and intense olfactory characteristics are known as essential oils. Chemically, essential oils can be terpenes, esters, alcohols, aldehydes, ketones, phenols, ethers, and hydrocarbons. Contributions from each individual class of compounds and quantitative proportions among these compounds determine therapeutic properties of a given essential oil [1].

Currently, a fast developing hybrid field formed at an intersection of natural medicine and the knowledge of medicinal plants is known as aromatherapy. Within its framework, the patient is treated with biologically active olfactory agents derived from herbal essential oils which penetrate his organism through the respiratory system and skin. Certain plants produce up to several hundred volatile compounds and many of those have never been properly identified yet [2].

This paper presents consecutive results derived within the framework of a complex research project on composition of essential oils originating from the different sage (*Salvia* L.) species. The analyses performed so far have enabled identification of 24 volatile compounds belonging to the class of terpenes [3, 4]. Presently, we introduce a combined evidence on chemical composition of essential oils obtained from the two different and essential-oil-rich sage species (i.e., from *Salvia lavandulifolia* L. and *Salvia triloba* L) by means of ^{13}C NMR spectroscopy and the *headspace*-GC-MS. This attempt is basically meant to gain an additional and a more in-depth perspective on composition of essential oils from the *Salvia* L. genus, owing to the complementary identification results derived by means of ^{13}C NMR spectroscopy that accompany thosee obtained by means of the *headspace*-GC-MS technique.

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The work of one author (Ł.W.) was partially supported by PhD scholarships granted to him in 2009 within the framework of the 'University as a Partner of the Economy Based on Science' (UPGOW) project, subsidized by the European Social Fund (EFS) of the European Union.

6.

Determination of lipophilicity by TLC revisited - a comparative study on several techniques with simple molecule model solute set

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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. While the problem is very comprehensively discussed in the case of HPLC, comparative studies by TLC were almost undone.

The purpose of the study was to compare several approaches of TLC lipophilicity determination: 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 RP18 thin layer plates and nine concentrations of six modifiers: acetonitrile, acetone, dioxane, propan-2-ol, methanol and tetrahydrofuran.

Comparative analysis formed several general recommendations:

1. Methanol and dioxane were the best modifiers, while acetonitrile gave the worst and inacceptable 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.
4. A need to use of robust regression and robust correlation measures, due to presence

7.

HPLC method determination of formaldehyde released from chosen root canal sealers

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The present investigation was concerned with determination of formaldehyde released from root canal filling and sealing materials. For the analysis four root canal sealers were chosen: EZ-Fill Endodontic Filling Cement, Endomethasone N Root Canal Sealer, AH Plus Jet Root Canal Sealing Material and Endodontic Cement N2. The cements samples (homogenous pastes) were prepared according to manufacturers' instructions.

Formaldehyde released from pastes was analyzed by RP - HPLC after derivatization with 2,4-dinitrophenylhydrazine and extraction with dichloromethane and acetonitrile. Analysis was performed by using a C18 chromatographic column, a mixture of acetonitryle and water (70:30) as a mobile phase, and UV/VIS detection (360 nm).

The standard curve was linear across the range 0,05 – 16 ppm of formaldehyde with a correlation coefficient 0,9996; the limit of detection was 0,01ppm. Precission (RSD) of the assay was 5,3% for pastes containing about 2000 ppm of formaldehyde and 6,9% for lower contents (about 2 ppm). Recovery studies were performed by fortyfying each paste with an amount of formaldehyde equal to that which was determined. The results were in the range 89% for pastes containing about 2000 ppm of formaldehyde and 76 – 86% for those containing about 2 ppm.

The results allow for application of this HPLC method for the analysis of formaldehyde released from the root canal sealers.

8.

Selective extraction of polar organic compounds as the key stage of chemical warfare metabolites identification in biomedical samples

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Biomatrices are the most challenging objects for investigation because of their complex and unpredictable qualitative and quantitative compositions. Biomatrices actively metabolize absorbed compounds even after their excretion from the body. Chemical warfare (CW) biotransformation products are usually polar and hydrophilic, which entails their high affinity to biomatrices and low recovery in standard extraction procedures.

Direct analysis of polar CW metabolites can be achieved by means of HPLC and CE techniques, but their sensitivity and selectivity are lower compared to GC. As participants of the OPCW Confidence Building Exercise on Biomedical Sample Analysis we had to develop optimal procedures for sample preparation for electron impact and chemical ionization GCMS analysis of *O*-alkylmethylphosphonic acids, thiodiglycol, and β-lyase metabolites of sulphur mustard. These procedures include extraction of target compounds from synthetic urine, clean-up and derivatization. Each sample should be tested for the presence of each analyte with minimum sample volume and time consumption and with a maximum reliability of identification. Sample preparation appeared to be a bottleneck.

Attempted SPE application was unsuccessful, probably due to a high concentration of artificially spiked masking agents. We have developed a universal procedure, involving purification of the synthetic urine by successive treatment with benzene and hexane in neutral and acid media. The purified synthetic urine was mixed with equal volume of acetonitrile, sodium chloride was added to the mixture, and the separated acetonitrile layer containing CW metabolites was separated (salt-assisted dispersive liquid-liquid extraction). The residual aqueous layer was extracted with acetonitrile and diethyl ether, the organic extracts were combined, concentrated and derivatized for subsequent GCMS analysis.

Thus we report here a procedure involving clean-up of synthetic urine by successive treatment with organic solvents, followed by salt-assisted dispersive acetonitrile extraction as a simple and reliable method for selective extraction of polar organic compounds from complex matrices.

9.

GC/MS analysis of the urine for metabonomic research of autistic children

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In combination with genomics, transcriptomics and proteomics, metabonomic analysis is being increasingly used in the clinical chemistry. Metabonomics is defined as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification". Application of metabonomics in clinical investigations is supported by a large number of publications. One of the aims of such research is discovering biomarkers, candidates specific for the condition, for assessing disease progression, determining drug efficacy, and potentially allowing selection of a patient. Urine, which is readily obtained, represents one of the most common sample types for this work but analysis can also be performed on other biofluids and secretions as well as whole tissues and tissue extracts. Combined techniques such as e.g. GC-MS, HPLC-MS, GC-TOF-MS are widely used in metabolic studies [1-4]. The use of GC-MS-based methods for the study of metabololite urinary profiles in research on autism can allow obtaining information on possible metabolic disorders in people affected by this disease. Autism is a developmental disorder, defined on the basis of behavioural characteristics which result from impairments in social communication and reciprocal social interaction, repetitive and restrictive behaviours, and imaginary thought [5].

The aim of this study was to perform metabolic profiling using GC-MS. The urine specimens were collected from 10 autistic children who underwent rehabilitation at the Navicula (Research Centre in Mental Retardation "Navicula" Centrum in Lodz, Poland) and 5 healthy children. The method involves extraction of analytes from urinary samples and derivatization with MSTFA.

This work was supported by grant from Polish Ministry of Science and Higher Education (No. NN 204 316234).

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10.

Urinary dicarboxylic acids in autism

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder diagnosed in early childhood. All children with ASD demonstrate deficits in social interaction, verbal and nonverbal communication, and repetitive behaviors or interests [1]. Several metabolic defects have been associated with autistic symptoms, these include phenylketonuria, histidinemia, adenylosuccinate lyase deficiency, dihydropyrimidine dehydrogenase deficiency, 5'-nucleotidase superactivity, and phosphoribosylpyrophosphate synthetase deficiency, mitochondrial dysfunction. Early diagnosis of the metabolic disorders and proper therapeutic interventions in some patients may significantly improve both cognitive abilities and behavioral deficiencies [2,3]. A very important tool used in the diagnosis of several metabolic disorders is the analysis of urinary dicarboxylic acids [4]. The chronic urinary excretion of dicarboxylic acid may be due to: 2-ketoglutarate degradation, ketosis, tissue ischemia (in case of succinate), ketosis, lactic acidosis; hypoglycemia, beta-oxidation defects HMG-CoA lyase deficiency, systemic carnitine deficiency, succinic semialdehyde DH deficiency, CPT II deficiency (in case of adipate, suberate, sebacate) uremia and peroxisomal diseases (in case of odd dicarboxylic acids) [5].

The aim of the study was to determine the level of succinic, adipic, suberic and azelaic acids in urine of autistic and healthy children. Urine samples were taken from 30 autistic children who underwent rehabilitation at the Navicula (Research Centre in Mental Retardation "Navicula" Centrum in Lodz, Poland) and from 10 healthy children. Before GC-MS analysis dicarboxylic acids were extracted from urine and derivatised with BSTFA.

This work was supported by grant from Polish Ministry of Science and Higher Education (No. NN 204 316234).

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11.

Determination of the level of D-arabinitol and D-/L-arabinitol ratio in urine of autistic children using gas chromatography/electrone capture detection

Wioletta Grys, Joanna Kałużna-Czaplińska, Joanna Gosławska, Jacek Rynkowski

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D-arabinitol is 5-carbon sugar alkohol (pentiol) is produced by many pathogenic yeast species. These include Candida albicans, Candida tropicalis, Candida parapsilosis, Candida kefyr, Candida lusitaniae and Candida guilliermondii. Human mammalian cells are capable of producing both D- and L-arabinitol. D-arabinitol is a well known marker for invasive candidasis. Clinical signs of invasive candidasis can be unspecific, and diagnosis is still mostly based on blood cultures; however, blood cultures have been assumed to be positive for Candida in only 24 to 60 % of cases. Both D-arabinitol and L-arabinitol are normally present in serum and urine, and the DA/LA ratio can be determined with coupled chromatography techniques. An elevated urine D-arabinitol/L-arabinitol (DA/LA) ratio is a sensitive sign of invasive candidasis.

Autism belongs to a group of disorders whose process can be modified by mycosis. Many symptoms of autism can be the result of pathological Candida's increase. Treating with antifungal drugs and application of gluten-free and casein-free diets can significantly improve clinical state of autistic subjects.

The aim of this work is to determine D-arabinitol and DA/LA ratio in urine of autistic children in comparison with healthy individuals using gas chromatography/electron capture detection (GC/ECD).

Literature: 1. Mycology 49 (2004), 117-123; 2. J Chromatogr B 773 (2002), 175-181;
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12.

Determination of the level of fumaric acid in urine of autistic children using SPE-HPLC

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Fumaric acid is the trans-isomer of malic acid that enters the citric acid cycle. It is necessary to generate cellular energy for tissue fuel. It is formed by oxidation of succinate by enzyme succinate dehydrogenase. Fumarate is then converted by the enzyme fumarase to malate. Fumaric acid is also a byproduct at certain stages in the arginine-urea cycle and purine biosynthesis. In healthy individuals, fumaric acid is formed in the skin from the exposure to sunlight. A deficiency in one or more Krebs' cycle intermediates and an inhibition of normal energy production may cause a wide range of metabolic disturbances and symptoms. A deficiency of fumaric acid is linked to chronic fatigue, psoriasis and fumarate hydratase deficiency, which is an autosomal recessive disorder of the Krebs cycle with variable presentation, usually involving developmental delay with neurological features such as epilepsy, hypotonia and encephalopathy. The commercial demand for fumaric acid has been increasing because of its extensive applications in the food industry. It is used in beverages and baking powders, as a substitute for tartaric acid and in place of citric acid.

There is evidence that some cases of autism have been associated with several different organic conditions, including bioenergetic metabolism deficiency. Cases of mitochondrial respiratory chain disorders have also been described as being associated with autism.

The aim of this work is to measure the level of fumaric acid in urine of autistic children in comparison with healthy individuals. Investigations were carried out with an application of high pressure liquid chromatography technique/ultraviolet detection (HPLC-UV) based on solid phase extraction method (SPE).

This work was supported by grant from Polish Ministry of Science and Higher Education (No. NN 204 316234)

Literature: 1. J Inherit Metab Dis 23 (2000), 757-759; 2. Develop Med Child Neurol 47 (2005), 185-189; 3. J Liq Chromatogr Related Technol 20 (1997), 3365-3376

13.

Urinary level of cysteine and mercury in autistic children

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Autism is heterogeneous neurodevelopmental disorders behaviorally defined by significant deficits in social interaction and communication. There are numerous theories concerning the specific causes of autism. One of these theories is exposure to toxic metals. In literature one can find suggestions that there are strong links between childhood autism and mercury toxicity (1-3).

The function of glutathione in human organism is connected with toxic metals and their removal. The cysteine thiol (-SH) group of glutathione binds mercury and protects essential proteins from functional inactivation. Mercury binds to cysteine thiol groups on intracellular proteins and inactivates their functions. A studies revealed the importance of transsulfuration metabolic imbalance present in many autistic children, characterized by significant reductions of cysteine in organism and reduced glutathione levels relative to controls (4,5).

The aim of the present study was to find out whether there are some tangible differences and correlation between the level of cysteine and mercury in urine of autistic children. Urine samples were collected from 30 autistic children (4-11 years) who underwent rehabilitation at the Navicula (Research Centre in Mental Retardation “Navicula” Centrum in Lodz).

Cysteine in urine was determined by gas chromatography/mass spectrometry (GC/MS). The total mercury content was measured with Mercury Analyser MERCURY SP-3D, Nippon Instrument Corporation.

This work was supported by grant from Polish Ministry of Science and Higher Education (No. NN 204 316234).

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14.

Determination of the level of homocysteine in urine of autistic children before and after a diet using gas chromatography/mass spectrometry

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Nutrition plays an important role in the development and behavior of autistic children. Autistic people have a high prevalence of gastrointestinal disease and dysbiosis. Improper diets and bad conditions of alimentary system can have a strong influence on the intensification of autism symptoms (1).

The level of homocysteine, which is eliminated with urine, provides essential data about diets and functioning of the alimentary system. Homocysteine is metabolised on two pathways: remethylation to methionine or transsulfuration to cysteine. A defect in either of these pathways leads to an accumulation of higher concentration of homocysteine in organism. Remethylation is a process which involves the presence of folic acid and vitamin B_{12}. Transsulfulation is a process which involves the presence of vitamin B_6. Vitamins B_6, B_{12} and folic acid are necessary for lowering the level of homocysteine. Improper dietary intakes of these nutrients can lead to vitamins deficiencies, which can produce elevations in homocysteine levels (2-4).

In our studies, we present the results of the level of homocysteine in the urine of autistic children before and after the diet enriched with vitamins from group B. Urine samples were collected from a group of autistic children (4-11 years) who underwent rehabilitation at the Navicula (Research Centre in Mental Retardation “Navicula” Centrum in Lodz). Gas chromatography/mass spectrometry (GC/MS) was used to determine the levels of homocysteine in urine.

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15.

Methods of determination selected priority substances 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, espiecially harmful effect on ecosystems and water of so called priority substances.

The aim of this work was to devise methods of determination selected priority substances, which were partitioned for three groups. Group I involving : di(2-ethylhexyl)phthalate, α,β-endosulfan, alachlor, chlorpyrifos, octylphenols and trifluralin was analyzed by GC/MS. For coumpounds: simazine, atrazine, diuron, isoproturon, chlorfenvinphos – group II, HPLC/DAD was chosen as a final method of determination.. Group III, represented by voltaic organic compounds: trichlorobenzenes, trichloromethane, benzene, 1,2-dichloroethane, dichloromethane was analyzed by SPME-GC-MS.

As a extraction technique of water samples for compounds from groups I and II Solid Phase Extraction (SPE) was applied comparing the two kinds of sorbents, C_{18} phase and copolymer SDB. For voltaic organic compounds - group III, Head Space-Solid Phase Microextraction (HS-SPME) was used.

The usefulness of the devised methods was estimated by comparing obtained limits of quantification of examined substances with limit values according to ordinance of the Ministry of Environment 20 August 2008 regarding the way of classification of surface water state (DZ. U. Nr 162, poz. 1008)

The devised methods were tested on natural environmental samples collected from areas with different contamination levels. Our researches have proved occurrence of priority substances in water which are situated in near neighborhood of industrial plants.

16.

Determination of nicotine in hair by GC-NPD

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It has been proved, that variety of xenobiotic can accumulate in hair during its growth. In addition, hair analysis belongs to noninvasive methods of assessment of exposure to tobacco smoke. Hair concentrations of nicotine was worked out by gas chromatography with nitrogen detector preceded by solvent extraction procedure.

Sixty milligrams of each hair portion were transferred to a polypropylene centrifuge tube for washing. Samples were washed using 6 mL of dichloromethane by sonication for 30 minutes to remove nicotine adhering to the surface of the hair. When the hair strands were dry after the washing step, 3mL of 1mol/L NaOH were added and the samples were incubated at 50°C for 24h, then cooled to room temperature. After incubation, 7 mL dichloromethane was added to the tubes. The tubes were finally shaken using centrifuged at 2000g for 10 minutes. The organic phase in each tube was then transferred to a clean polypropylene evaporation tube. To prevent volatilization of nicotine, 35 μL n-octanol was added to each tube. The extraction process was repeated and the organic layers from each extractions were evaporated together in a water bath at 50°C for approximately 20 minutes. Finally, methanol was added to the remaining of octanol and the final solution was transferred to an insert mounted in an autosampler vial and analysed by GC-NPD (Shimadzu, Japan) with ZB-5 (30m x 0,25mm x 0,50μm) column. Temperature programme: 50°C (for 1min); 50°C to 215°C (10°C/min for 1 min); 215°C to 275°C (20°C/min) for 2 min) was used. Detector and injector temperatures were 300°C and 250°C, respectively. Helium and flow rate of 2 cm^3/min was applied as carrier gas.

The main nicotine metabolites - cotinine can be determined in much more concentrations, but 3-trans'-hydroxycotinine should be derivatised before gas chromatographic determination. Therefore we were only interested in the measurement of nicotine with detection limit of 0.15 ng/μL, determined by GC-NPD.

Moreover, n- octanol addition does not improve nicotine recovery from hair. The worked out procedure can be also apply in nicotine replacement therapy(NRT).

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17.

Determination of lipophilicity of tritolylporphyrin derivatives using TLC

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The worldwide laboratories are working to develop a new, effective methods for combat diseases, particularly cancer. One of the developing methods of the fight against cancer is Photodynamic Therapy, which is based on the use of photosensitizers, amoung other for this reason can be used the porphyrin rings.

One of the parameters, which allows to determine the biological activity of some compound is its lipophilicity. Lipophilicity can also be determined by TLC (*thin-layer chromatography*) on reversed phase, in addition to the traditional method of determining this parameter (which is the *shake-flask method*). In presented study, lipophilicity was determined for carboxyalkyl derivatives of tritolylporphyrin, which can potentially be used as the photosensitizers in Photodynamic Therapy.

18.

Lipophilicity of new potential photodynamic therapy agents

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Photodynamic therapy (PDT) is quickly developing method for the treatment of different kinds of cancer diseases based on possibility of light interaction with chemical compounds. However every medical application of new chemical compounds is faced with delivery problems into the organism. Very important feature of photosensitizer good for photodynamic therapy is its selectivity to pathological tissue. Attractive method for delivery and increasing selectivity seems to be the possibility of placing the photosensitizer into liposoms.

Lipophilicity is an important parameter which allows foreseeing of biological activity or the accumulation of drugs in the organism, especially their penetration into cell membranes. Thin layer chromatography as an alternative to the traditional *shake-flask* method is a quick and convenient way for determination of lipophilicity. In this work we assigned lipophility for series of new derivatives of tetra(hydroxyphenyl) porphyrins as potential agents in PDT with use of reversed phase thin layer chromatography.