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Posters

Sinan Cavun et al., Biochem Pharmacol (Los Angel) 2018, Volume 7
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Glycyl-glutamine: A new hope in the treatment of depression?

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Statement of the Problem: Depression is the 4th leading cause of disability and death all around the world and it is expected to be be the 2nd cause after 2020. Globally, more than 300 million people of all ages suffer from depression. The high rate of patients who still have not responded to depression treatment makes it necessary to perform preclinical research. The current medications used in depression treatment are also very troublesome drugs in respect of the adverse effects they cause. The risks and adverse effects of antidepressants currently used for depression treatment spread in a large range from sexual problems to death. In the current brain microdialysis studies from our laboratory, it is shown that glycyl-glutamine (Gly-Gln) augments serotonin release in the brain. It is well known that serotonin is a hormone that makes people feel happy, energetic and lively. In this study, we aim to investigate antidepressant effects of Gly-Gln dipeptide because of its enhancing effects on serotonin levels.

Methodology: These studies have been performed by the "forced swimming test" method which is the most applied animal model used in antidepressant treatment surveys. Using the swimming test, the dose response study, comparison with Gly-Gln cleavage products and fluoxetine, as well as locomotor activity test was performed.

Findings: In this sense, animal studies performed with "glycyl-glutamine" showed that it is tremendously effective against depression.

Conclusion: The most important feature of Gly-Gln is being a molecule, which can be synthesized in our body endogenously, and it is comes off while β-endorphin is being burned in the body. Therefore, Gly-Gln is extremely safe with its adverse effects. There isn't any adverse effect observed during the toxicological studies. This result is important for considering Gly-Gln as a potential antidepressant.

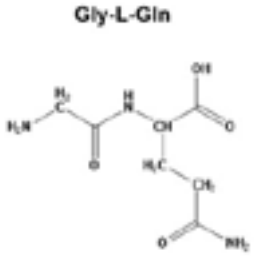

Figure 1: Chemical composition of glycyl-glutamine.

Recent Publications

1. M Guclu, S Kiyici, Z Gul and S Cavun (2017) Exenatide treatment causes suppression of serum fasting ghrelin levels in patients with type 2 diabetes mellitus. Endocrine Connections EC-17-0242.
2. N F Basaran, R L Buyukuysal, M S Yilmaz, S Aydin, S Cavun, et al. (2016) The effect of Gly–Gln [ß-endorphin 30–31] on morphine-evoked serotonin and GABA efflux in the nucleus accumbens of conscious rats. Neuropeptides 58:23–29.
3. S Kiyici, N F Basaran, S Cavun and V Savci (2015) Central injection of CDP-choline suppresses serum ghrelin levels while increasing serum leptin levels in rats. European Journal of Pharmacology 764:264–270.
4. N F Basaran, R L Buyukuysal, W R Millington and S Cavun (2010) Glycyl-
5. glutamine (β-endorphin30-31) inhibits morphine-induced dopamine efflux in the nucleus accumbens. Naunyn-Schmiedeberg's Archives of Pharmacology, 381(5):467–475.

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Biography

Sinan Cavun, after graduating from the Uludag University, Faculty of Medicine in 1993, completed his Doctoral Education in 1999. He has been working in the field of Pharmacology for a total of 17 years. He worked for eight years in Hospital Medicine Management as a Team Leader. He has focused on neuropharmacology, particularly on depression and neuroendocrine regulation. As a result of his work, he has a European patent in this regard (use of Gly-Gln in the treatment of depression).

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Richard L Summers et al., Biochem Pharmacol (Los Angel) 2018, Volume 7
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Role of biobanking in discovering pharmacologic biomarker targets for the practice of precision medicine

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Statement of the Problem: Clinical studies that examine the outcomes of different treatments have guided the development of standards of care for the practice of medicine. While the evidences of these clinical trials has led to an improved practice of medicine, they focus on the outcome likelihoods for the population of patients as a whole and therefore treatment guidelines have evolved into a one-size-fits-all methodology. Recently, the modern methods of precision/personalized medicine have emerged as the course of medical practice for the future. The discovery of biomarker targets for the pharmacologic strategies of precision medicine will be critical to the development of this practice.

Methodology: Developing precision/personalized medicine systems will require an improved capacity to link biologic biomarkers with basic clinical, demographic and socioeconomic phenotypes. Such biomarkers can then be used as targets for patient-specific pharmacologic treatment strategies. A process that back tracks clinical treatment outcomes or adverse events to biologic materials banked in a prospectively instituted biorepository system can be used in the discovery of these potential biomarker targets.

Results: A software interface was used to link information from the electronic health record (Epic) and other salient data contained within the enterprise data warehouse with samples in the biobank at the University of Mississippi Medical Center. Researchers analyzing the epidemiologic characteristics of the data warehouse can identify cohorts of patients with specific responses to prescribed pharmacologic treatments. The biobanked biologic specimens that are associated with the individuals in these cohorts can then be recovered for an analysis of their pharmacogenetics and other omics as potential biomarker targets.

Conclusion: The new precision medicine approach to clinical practice represents a shift in the philosophy of treatment schemes that will require a greater focus on the translation of basic biomedical research to practical patient applications. Linking biobanked specimens to observed outcomes in clinical cohorts will be critical to the discovery of biomarker targets that facilitate decisions regarding precision pharmacologic strategies.

Recent Publications

1. Pauli C, Moch H and Rubin M A (2017) Establishment of a living biobank: improved guidance of precision cancer care with *in vitro* and *in vivo* cancer models. Pathologe. 38(2):160–168.
2. Ostrom Q T, Devine K, Fulop J, Wolinsky Y, Liao P, et al. (2017) Brain tumor biobanking in the precision medicine era: building a high-quality resource for translational research in neuro-oncology. Neurooncol Pract. 4(4):220–228.
3. Vaught J (2016) Biobanking comes of age: the transition to biospecimen science. Annu Rev Pharmacol Toxicol. 56:211–28.

4. Somiari S B and Somiari R I (2015) The future of biobanking: a conceptual look at how biobanks can respond to the growing human biospecimen needs of researchers. Adv Exp Med Biol. 864:11–27.

Biography

Richard L Summers, MD, FACEP is Associate Vice Chancellor for Research, University of Mississippi Medical Center Billy S Guyton Distinguished Professor and Chair Emeritus Department of Emergency Medicine Previous Lead Scientist for NASA Digital Astronaut Project. Richard L Summers, MD, FACEP is a native of Gulfport Mississippi and graduated from the University of Southern Mississippi magna cum laude in mathematics in 1977. He received his medical degree from the University of Mississippi Medical Center in 1981 after which he entered their residency program in internal medicine. Summers then began graduate studies and completed a research fellowship under Dr. Arthur C Guyton and Dr. Thomas G Coleman in the Department of Physiology and Biophysics. Since 1988 he has been a faculty member at the University of Mississippi Medical Center in various roles including Chairman of the Department of Emergency Medicine. He currently serves as the Associate Vice Chancellor for Research and holds joint appointments in the Department of Emergency Medicine and the Department of Physiology and Biophysics and is a Fellow of the American College of Emergency Physicians.

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Flaszka E et al., Biochem Pharmacol (Los Angel) 2018, Volume 7
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Role of melatonin in the prevention of noise-induced hearing loss

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Melatonin is a hormone produced by the pineal gland in animals that regulates sleep and wakefulness. It is involved in the circadian rhythms synchronization including sleep-wake timing, blood pressure regulation, seasonal reproduction, etc. However, other physiological roles have been described for melatonin such as antioxidant protection of nuclear and mitochondrial DNA, anti-inflammatory effect by TNF-α inhibition, etc. Because increasing number of studies demonstrated that antioxidants may serve as effective compounds to block cochlear inflammation and hair cells apoptosis, targeting members of antioxidant pathways could be feasible options for the treatment of several types of hearing loss. For this reason, the aim of this study is to determine the effect of melatonin in the hearing impairment and anxiety induced by acoustic trauma.

Figure 1: Role of melatonin in the prevention of noise induced hearing loss.

Recent Publications

1. Casteil, et al. (2017) Study of the efficacy of melatonin for auditory brainstem response (ABR) testing in children. European Annals of Otorhinolaryngology, Head and Neck Diseases 134.
2. Tao, et al. (2009) Protective effect of melatonin against gentamicin ototoxicity. The Journal of Laryngology & Otology 123:598–602.
3. Bas, et al. (2009) An experimental copmparative study of dexamethasone, melatonin and tacrolimus in noise-induced hearing loss. Acta Oto-Laryngologica 129:385–389.
4. Lopez-Gonzalez, et al. (2000) Ototoxicity caused by aminoglycosides is ameliorated by melatonin without interfering with the antibiotic capacity of the drugs. J. Pineal Res. 28:26–33.
5. Lopez-Gonzalez, et al. (1997) Presence of the pineal hormone melatonin in rat cochlea its variations with lighting conditions. Neuroscience Letters 238:81–83.

Biography

Elodie demonstrates an excellent level of technical skills in fields including neurosurgery, general surgery, and behavioral tests. A graduate in analytical and experimental biology, she has been working for 5 years on the development of drug candidates in neurosciences. For CILcare, she specializes in the in-vivo models of ear inflammation, and carries out efficacy studies, as well as other tasks, from designing protocols to interpreting discovery data, with great talent and dynamism

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Bayçu C et al., Biochem Pharmacol (Los Angel) 2018, Volume 7
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Is mercury inhalation triggering the NF-KB pathway in the lungs? A histochemical study in rat model

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Statement of the Problem: Mercury is an element that is available in batteries, thermometers, barometers, light bulbs by inorganic form and also in pillars; fish, mussels and some seafood by organic form. It is visible in tooth fillings used in dental treatment, in certain vaccinations and also in antiseptic solutions. It is thought that mercury vapor released from all these compounds is harmful for human health. NF-κB is a silent factor in the cytoplasm of all cells and passes to the nucleus only when it is active, where it regulates the immune system growth and inflammation (Figure 1). The object of the study is to detect the immunohistochemical expression levels of the nuclear factor kappa B (NF-kB) in the mercury vapor exposed rats and evaluate the possible association between the expression levels and histological structure of the lung.

Methodology & Theoretical Orientation: In this study, 12 adult Wistar albino rats (12 female, weighing 200 g) were used for the above-mentioned purpose. The rats were placed in specially designed lantern. Following this procedure, subjects were exposed to 1 mg/m3/day of mercury vapor for 45 days. Subjects who did not have any application were used for control group. At the end of the experiment, lung samples obtained from all subjects were followed up according to routine procedure and evaluated histologically at light microscopic levels and immunohistochemically with NF-κB marker.

Findings: In the lungs of the subjects exposed to mercury vapor, alveolar edema and enlarged inter alveolar connective tissue septa were found. Erosion of bronchial epithelium and mononuclear cell infiltration on the wall of the conductive structures occurred. The alveoli in these lung specimens were collapsed. In addition, NF-κB immunostaining in lung tissues of exposed to mercury vapor had increased significantly.

Conclusion & Significance: Although the mercury element is liquid at normal room temperature, it evaporates after a certain period of time, causing humans to suck up from the lungs as a result of the breathing of the air. In this study, it has been shown that chronic exposure to mercury vapor may cause lung damage and NF-KB pathway may play a role in this damage.

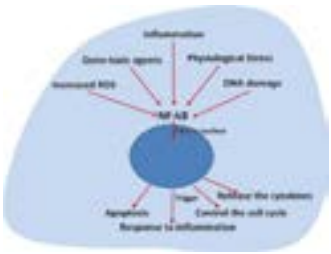

Figure 1: Regulation of the cell functions by the activity of the NF-kB.

Recent Publications

1. Yahyazedeh A, Altunkaynak B Z, Akgül N and Akgül H M (2017) A histopathological and stereological study of liver damage in female rats caused by mercury vapor. Biotech Histochem. 92(5):338–346.
2. Akgül N, Altunkaynak B Z, Altunkaynak M E, Deniz Ö G, Ünal D, et al. (2016) Inhalation of mercury vapor can cause the toxic effects on rat kidney. Ren Fail. 38:465–473.
3. Altunkaynak B Z, Akgül N, Yahyazadeh A, Altunkaynak M E, Turkmen A P, et al. (2016) Effect of mercury vapor inhalation on rat ovary: stereology and histopathology. J Obstet Gynaecol Res. 42(4):410–416.

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5. Beg A A and Baltimore D (1996) An essential role for NF-κB in preventing TNF-α-induced cell death. Science 274:782–784.

Biography

Bayçu C is Professor in Histology and Embryology. He has his expertise in toxicological studies in the animal models. Also, he studies on new pathways for improving healthcare. He uses light and electron microscopy and also histochemical and molecular techniques for his studies.

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B Zuhal Altunkaynak et al., Biochem Pharmacol (Los Angel) 2018, Volume 7
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The effects of topiramate on liver and NPY levels in female obese rats: Stereological and histopathological study

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[2]Ondokuz Mayıs University, Turkey
[3]Karabük University, Turkey

Statement of the Problem: Obesity, defined as one of the 10 most risky diseases by the World Health Organization, affects almost all the system and organs in the body. Topiramate (TOP) is an antiepileptic drug which has also been proved to alleviate body weight. Neuropeptide Y (NPY) is a 36 amino-acid neuropeptide that is involved in various physiological and homeostatic processes in both the central and peripheral nervous systems (Figure 1).In this study, we aimed to develop an obesity model on adult rats with a high fat diet and to investigate the possible effect of TOP on liver in the high-fat-diet (HFD)-induced obese female rats and whether these possible effects are related to NPY levels by histological and morphometric methods.

Methodology & Theoretical Orientation: For this aim, 24 female Wistar albino rats were randomly divided in four equal groups, viz. control (CONT), obese (OBES), TOP, and OBES+TOP. Following processing and cutting the liver tissue, sections were used for histopathological examination and stereological analyses. Also immunohistochemical analyses were made by NPY marker.

Findings: Given the stereological outcomes, total number of hepatocytes was reduced in the OBES+TOP group compared to those of the TOP group. In terms of the mean sinusoid volume, no meaningful difference was distinguished among the groups. Likewise, histopathological findings exhibited mild to severe alterations in the manifestation of liver architectures in experimental rats (OBES, OBES+TOP, TOP). While NPY positivity increased in obese rats, it decreased in TOP administrated groups.

Conclusion & Significance: In conclusion, our findings presented that TOP administration associated with obesity decreases body weight by setting the NPY level. For all that, it may have deleterious influence on the liver tissue in the subjects and hepatocyte loss might be derived from the possible side effect of TOP in combination with obesity. Hence, the both are risk factors enhancing hepatotoxicity.

Figure 1: Homeostatic mechanism induced by NPY.

Recent Publications

1. Lee J S, Jun D W, Kim E K, et al. (2015) Histologic and metabolic derangement in high-fat, high-fructose, and combination diet animal models. Scientific world Journal 306326.
2. Alkan I, Altunkaynak BZ, Altun G and Erener E (2017) The investigation of the effects of topiramate on the hypothalamic levels of fat mass/obesity-associated protein and neuropeptide Y in obese female rats. Nutr Neurosci. 15:1-10.
3. Tüfek NH, Altunkaynak ME, Altunkaynak BZ and Kaplan S (2015) Effects of thymoquinone on testicular structure and sperm production in male obese rats. Syst Biol Reprod Med. 61(4):194-204.

4. Masarone M, Federico A, Abenavoli L, et al. (2014). Nonalcoholic fatty liver: epidemiology and natural history. Rev Recent Clin Trials 9:126-133.
5. Bekar E, Altunkaynak BZ, Balcı K, Aslan G, Ayyıldız M and Kaplan S (2014) Effects of high fat diet induced obesity on peripheral nerve regeneration and levels of GAP 43 and TGF-β in rats. Biotech Histochem. 89(6):446-56.

Biography

B Zuhal Altunkaynak is a Professor in Histology and Embryology. She has expertise in studies about pathophysiology and neural pathways of obesity and their treatment in the animal models. She uses light and electron microscopy and also histochemical and molecular technics for these studies.

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Teodor Angelov et al., Biochem Pharmacol (Los Angel) 2018, Volume 7
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Ophthalmic infection with *Dirofilaria repens* in Bulgaria

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[1]Medical University of Sofia, Bulgaria
[2]Eye Clinic Zrenie, Bulgaria

Introduction: Dirofilariasis is a parasitic disease of domestic and wild animals, that rarely infects humans. The genus Dirofilaria belongs to the family Onchocercidae and subfamily Dirofilariinae of the order Spirurida. It infects different mammals such as dogs, cats, foxes, etc. The parasite replicates in the animal's body and enters circulation in the form of microfilariae. Mircrofilariae are transmitted to humans through biological vectors such as certain species of mosquitoes. In fact *Dirofilaria sp.* rarely infects humans. The parasite is found in subcutaneous tissue and mucous membranes, rarely affects visceral organs – heart, lungs, eyes, central nervous system. Ophthalmic infection with *D. repens* is registered all over the world. Ocular involvement may be periorbital, subconjunctival or intraocular.

Materials and Methods: We describe a case of subconjunctival dirofilariasis in 64-year-old female patient, hospitalized in the Clinic of Ophthalmology. The 64-year-old patient presented with redness, irritation, intermittent local pain of the left eye. Her complaints dated from 3 months. Results: Ophthalmic examination revealed a thin white live worm under the chemotic and injected bulbar conjunctiva . The parasite was removed surgically under local anaesthesia. It was long 130 mm and wide 0.61 mm. The worm was identified as *Dirofilaria repens* in The Center of Parasitology, Sofia, Bulgaria.

Discussion: Cases of *D. repens* have been reported in Mediterranean Basin (Greece, Italy, Spain) and Turkey. The first case was published in 1867 by Angelo Pace in Palermo. Most cases present with pain, redness of the eye and swelling. Symptoms appear when worm enters the subconjunctiva – usually weeks after infection. Diagnosis should include: blood smear elavuation for microfilaria, serology, PCR-teston mosquitos to detect microfilarial DNA. In the literature in the biggest part of the described cases , the parasites length is 40-140 mm. In our case the parasite was 130 mm in length. Laboratory tests showed negative blood eosinophilia.

Conculsion: Ocular dirofilariasis, caused by *Dirofilaria repens* is very rare in Bulgaria. Most of the cases were registered in Asia and Africa, rarely in Central and South Europe. There are about 780 cases reported in the literature to date.

Biography

Teodor Angelov is 4th year student in Medical University of Sofia. Since his preclinical years in the University, he took part in many student congress as an active participant. Teodor has got many publications in bulgarian science journals in bulgarian and english. His interests are concentrated in neurology and neuroophthalmology, cognition sciences and degenerational diseases of the brain, morphology of the human. Teodor finds the participation in the Pharmacology congress for a big experience in his science bio and wants to create new contacts with students and professors in the Pharmacology area

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Behnam Ghorbanzadeh et al., Biochem Pharmacol (Los Angel) 2018, Volume 7
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Local antinociceptive action of fluoxetine in the rat formalin assay: Role of L arginine/nitric oxide/cGMP/KATP channel pathway

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The present study was conducted to evaluate the local antinociceptive actions of fluoxetine, a selective serotonin reuptake inhibitor, and the possible involvement of the L-arginine/NO/cGMP/K_{ATP} channel pathway in this effect using the formalin test in rats. To elucidate the underlying mechanisms, animals were pre-treated with L-NAME, aminoguanidine, methylene blue, glibenclamide, L arginine, sodium nitroprusside, or diazoxide. Local ipsilateral, but not contralateral, administration of fluoxetine (10–300 mcg/paw) dose-dependently suppressed flinching number during both early and late phases of the test, and this was comparable with morphine, also given peripherally. Pre-treatment with L-NAME, aminoguanidine, methylene blue, or glibenclamide dose-dependently prevented fluoxetine (100 mcg/paw)-induced antinociception in the late phase. In contrast, administration of L-arginine, sodium nitroprusside, and diazoxide significantly enhanced the antinociception caused by fluoxetine in the late phase of the test. However, these treatments had no significant effect on the antinociceptive response of fluoxetine in the early phase of the formalin test. Our data demonstrates that local peripheral antinociception of fluoxetine during the late phase of the formalin test could be due to activation of L-arginine/NO/cGMP/KATP channel pathway. The peripheral action of fluoxetine raises the possibility that topical application of this drug (e.g., as a cream, ointment, or jelly) may be a useful method for relieving the inflammatory pain states.

Biography

Behnam Ghorbanzadeh completed his PhD in Pharmacology at Ahvaz Jundishapur University of Medical Sciences in Iran. He started Research in Pharmacology in 2010 and has a strong research focus on Neuropharmacology and behavior in animal models. He currently works as an Assistant Professor in Pharmacology at the Department of Pharmacology, School of Medicine, Dezful University of Medical Sciences, Dezful, Iran.

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Sayaka Hashimoto et al., Biochem Pharmacol (Los Angel) 2018, Volume 7
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Atomic and isomeric high-resolution separation of thiolate-protected alloy clusters by reversed-phase high-performance liquid chromatography

Sayaka Hashimoto and **Yuichi Negishi**
Tokyo University of Science, Japan

By doping heteroatoms to thiolate-protected gold clusters, we can add more functionality to the cluster depending on the number of doped heteroatoms and the difference in doping positions. However, in the synthesis of alloy clusters, the mixture of clusters with a different number of heteroatoms is produced. Therefore, a precise separation of each cluster is required to understand their properties. In this study, we attempted to separate gold-silver alloy clusters precisely, furthermore investigated isomer change by reversed-phase high-performance liquid chromatography (RP-HPLC). $Au_{38-n}Ag_n(SC_4H_9)_{24}$ was used as a sample. This cluster was synthesized by two methods, (1) by adding $[Ag(SC_4H_9)]$ complex to $Au_{38}(SC_4H_9)_{24}$ (metal exchange) and gold and silver ions were reduced in the presence of butanethiol in solution (co-reduction). The mixture of alloy clusters was separated by RP-HPLC using gradient program for controlling mobile phase, and core-shell type column. The peaks obtained from the chromatogram were evaluated by electrospray ionization (ESI) mass spectrometry connected to RP-HPLC directly. Figure.1 (a) shows the chromatogram of $Au_{38-n}Ag_n(SC_4H_9)_{24}$ obtained by the metal exchange. Some clear peaks were observed in chromatogram. Each peak was attributed to the cluster having a precise number of silver atoms (Figure. 1(b)). These results indicate that the mixture of $Au_{38-n}Ag_n(SC_4H_9)_{24}$ was precisely separated according to the number of silver atom. Furthermore, the shape of chromatogram of $Au_{38-n}Agn(SC_4H_9)_{24}$ prepared by the metal exchange changed by leaving this cluster in toluene for 6 days (Figure. 1(c)). Interestingly, the shape was similar to that of $Au_{38-n}Ag_n(SC_4H_9)_{24}$ prepared by the co-reduction (Figure. 1 (d)). These results suggest that $Au_{38-n}Ag_n(SC_4H_9)_{24}$ prepared by the metal exchange contains metastable clusters, these are transformed to the stable clusters by leaving in toluene. In conclusion, we have succeeded in the high-resolution separation of alloy clusters according to each chemical composition, and observation of isomer transformation.

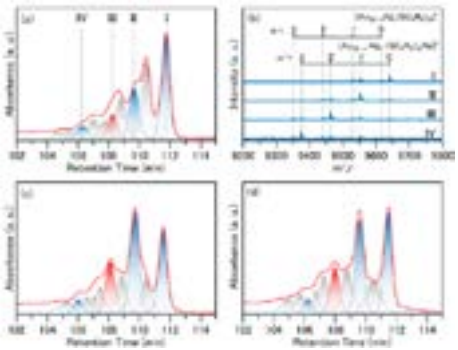

Figure 1: (a) Chromatograms obtained by metal exchange, (b) ESI spectrum of each peak in chromatogram, (c) Chromatograms obtained by metal exchange after 6 days, and (d) Chromatograms obtained by co-reduction.

Recent Publications

1. Y Negishi, et al. (2016) Precise synthesis, functionalization and application of thiolate-protected gold clusters. Coord. Chem. Rev., 320-321:238-250.
2. Y Negishi, et al. (2016) High-resolution separation of thiolate-protected gold clusters by reversed-phase high-performance liquid chromatography. Phys. Chem. Chem. Phys. (Perspective) 18:4251-4265.
3. Y Negishi, et al. (2015) Understanding ligand-exchange reactions on thiolate-protected gold clusters by probing isomer distributions using reversed-phase high-performance liquid chromatography. ACS Nano 9: 9347-9356.

4. Y. Negishi, et al. (2015) A critical size for emergence of nonbulk electronic and geometric structures in dodecanethiolate-protected au clusters. J. Am. Chem. Soc. 137:1206-1212.
5. Y. Negishi, et al. (2013) Separation of precise compositions of noble metal clusters protected with mixed ligands J. Am. Chem. Soc. 135:4946-4949.

Biography

Sayaka Hashimoto received her BSc in Applied Chemistry at Tokyo University of Science, Japan, in 2018. Her main research interest and work includes "Establishment of high resolution-separation technique of reversed-phase high-performance liquid chromatography (RP-HPLC)". She has recently succeeded in separating gold-silver alloy cluster by improving the resolution of RP-HPLC.

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Kana Yoshida et al., Biochem Pharmacol (Los Angel) 2018, Volume 7
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Precise separation of gold clusters protected by hydrophilic thiolates and evaluation of their chemical compositions by LC/MS

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Tokyo University of Science, Japan

Small thiolate-protected gold clusters show size-specific physical and chemical properties, which are not observed in bulk gold. Especially, hydrophilic thiolate-protected gold clusters exhibit a good affinity for biomaterials. Therefore, much research has been conducted in this field. However, it is difficult to selectively synthesize the clusters with specific chemical composition. Therefore, to evaluate properties of hydrophilic thiolate-protected gold clusters accurately, we need to separate single cluster from the mixture of these clusters with high resolution. In this work, we report on precise separation of various hydrophilic thiolate-protected gold clusters (Aun(SR)m, SR=SG (glutathionate), NALC (N-acetyl-L-cysteine), p-MBA (p-mercaptobenzoic acid)) by hydrophilic interaction liquid chromatography (HILIC). Furthermore, we attempted to evaluate the chemical composition of each cluster by introducing LC/MS which was directly connected the chromatograph with the mass spectrometer. The mixture of clusters used in this work was synthesized by reducing gold ions in the presence of thiols. Figure 1(a) shows the UV chromatogram of Aun(SG)m. Multiple peaks were observed in the chromatogram. Figure 1(b) shows the ESI-mass spectrum of each peak observed in the chromatogram. It was found that almost only one cluster was contained in each peak. These results indicate that mixture was separated with high resolution according to the chemical composition of clusters. Similar separation has also been achieved for Aun(NALC)m and Aun(p-MBA)$_m$. Therefore, it was revealed that the use of HILIC columns is powerful tool for separating of gold clusters protected by many kinds of hydrophilic thiolates. Furthermore, Although chemical compositions observed for Aun(SG)m and Aun(NALC)m were similar each other, that for Au$_n$(p-MBA)$_m$ was less compared with these clusters. These results indicate that chemical compositions of the Aun(SR)m vary depending on the ligand structure. In this manner, we have succeeded in revealing how difference of ligand affects synthesis of gold clusters protected by hydrophilic thiolate ligands.

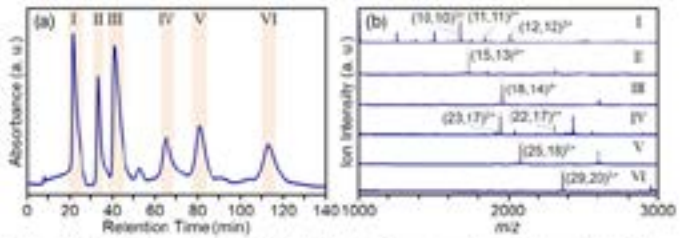

Figure 1: (a)UV chromatogram of Au$_n$(SG)$_m$. (b)Mass spectra of each peak (I–VI). The notation $(n, m)^{z+}$ indicates $[Au_n(SG)_m+zH]^{z+}$ for n = 10–12, 15, 18, and 23, whereas it indicates $[Au_n(SG)_m+(z+1)H]^{z+}$ for n = 22, 25, and 29 because of the negative charge of the metal cores of $Au_{22}(SG)_{17}$, $Au_{25}(SG)_{18}$, and $Au_{29}(SG)_{20}$.

Figure 1: (a) Chromatograms obtained by metal exchange, (b) ESI spectrum of each peak in chromatogram, (c) Chromatograms obtained by metal exchange after 6 days, and (d) Chromatograms obtained by co-reduction.

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5. Y Negishi, et al. (2014) Advanced use of high-performance liquid chromatography for synthesis of controlled metal clusters. *Nanoscale* 6:7889-7896.

Biography

Kana Yoshida has completed her BSc in 2017 in Applied Chemistry at Tokyo University of Science, Japan. Her main research interests are high resolution separation of noble metal clusters protected by hydrophilic thiolate ligands. She has presented her work many times at conferences. Furthermore, her research was published to *Nanoscale* (IF=7.367) which is one of the high impact factor journal in nanoscience field.

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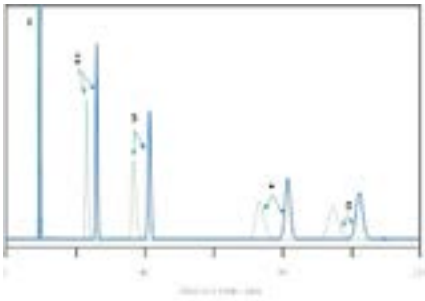
Updating chromatographic predictions by accounting ageing for single and tandem columns

Tamara Alvarez-Segura, José Ramón Torres-Lapasió and **María Celia García-Alvarez-Coque**
University of Valencia, Spain

Model-based chromatographic optimizations provide the best separation conditions efficiently. Unfortunately, with the routine usage, columns inevitably suffer some deterioration and, as a result, actual chromatograms may differ from the forecasted expectancies; new optimal separation conditions are then needed, but unfortunately, models are not valid anymore. The chromatographer could then decide repeating the whole modelling process, but this solution is undesirable and too time consuming, particularly when several coupled columns are involved. We propose a shortcut to correct time and peak profile predictions. The original models are corrected by introducing parameters accounting the deterioration, obtained using a small subset of compounds, selected from those studied in the training set before the column had suffered performance decline. The ageing parameters are not solute dependent. These are fitted in such a way that they minimize the discrepancies between the data predicted with the original retention models for the brand-new column and the experimental data measured for the aged column. The approach was developed and tested to predict the chromatographic behaviour of 15 sulphonamides, analysed with individual and tandem columns, using isocratic and gradient elution. From the gathered information, predictions more in line with the deteriorated performance were forecasted for the whole family of compounds analysed with that column (or set of coupled columns). The results suggest that, whenever the columns keep sufficient performance, we can take advantage of the extensive experimental work carried out when the system was initially modelled with the brand-new columns. With this information and a minimal extra experimental effort, accurate enough predictions in the degraded situation are possible. The full modelling of the chromatographic behaviour is thus only made with the brand-new columns. The agreement between predicted and experimental chromatograms in the aged columns was excellent.



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4. Ortiz-Bolsico C, Torres-Lapasió JR and García-Alvarez-Coque MC (2014) Optimization of gradient elution with serially-coupled columns: Part I: Single linear gradients. Journal of Chromatography A 1350:51-60.

5. Ortiz-Bolsico C, Torres-Lapasió JR and García-Alvarez-Coque MC (2014) Optimization of gradient elution with serially-coupled columns: Part II: Multi-linear gradients. Journal of Chromatography A 1373:51-60.

Biography

Tamara Alvarez-Segura completed her Master degree in Experimental Techniques in Chemistry in 2014, at the University of Valencia. She is now performing diverse research activities in the Department of Analytical Chemistry to get her PhD degree. She has received two Pre-doctoral fellowships. During her Master studies, she began her collaboration with María Celia García-Alvarez-Coque and José Ramón Torres-Lapasió in the field of the modulation of the selectivity in HPLC, using serially coupled columns and other strategies to analyse complex samples. She has written 11 research articles and presented several communications in international meetings. The PhD period included a three-month stay (September to November 2017) in the Analytical Chemistry Department at the University of Barcelona (Spain), under the supervision of Prof Martí Rosés, working in the field of Hydrophilic Interaction Liquid Chromatography (HILIC).

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Application of serially-coupled columns in chiral liquid chromatography

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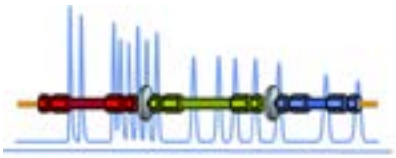
Liquid chromatography with single columns often does not succeed in the analysis of complex samples, in terms of resolution and analysis time. A relatively simple solution to enhance chromatographic resolution is the modulation of the stationary phase through the serial coupling of columns. This can be implemented with any type of column using compatible elution conditions and conventional instruments. This review describes the key features of column coupling and published procedures, where two or more columns were coupled in series to solve separation problems in chiral liquid chromatography. In most reports, two chiral columns are coupled. However, coupling chiral and achiral stationary phases is also an active field, to analyze samples containing mixtures of chiral and achiral compounds. In all reports, the authors could not resolve their samples with single columns, whereas significant enhancement in chromatographic performance was obtained when the columns were combined. Particularly interesting is the reduction in the analysis time in the isocratic mode, which alleviates the general elution problem of liquid chromatography, and may represent a stimulus for the proposal of new procedures, especially in combination with mass spectrometric, electrochemical and refractometric detection. Developments proposed to make the serial coupling of columns useful in routine and research laboratories are outlined, including optimisation strategies that facilitate the selection of the appropriate column combination and elution conditions (solvent content, flow rate or temperature) in both isocratic and gradient modes. The availability of zero dead volume couplers, able to connect standard columns, and the commercialisation of short columns with multiple lengths, has expanded the possibilities of success.



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Biography

Tamara Alvarez-Segura has completed her Degree in Chemistry in 2013 and Master degree in Experimental Techniques in Chemistry in 2014, both at the University of Valencia. She is now performing diverse research activities in the Department of Analytical Chemistry to get her PhD degree. She has received two pre-doctoral fellowships. During her Master studies, she began her collaboration with María Celia García-Alvarez-Coque and José Ramón Torres-Lapasió in the field of the modulation of the selectivity in HPLC, using serially coupled columns and other strategies to analyse complex samples. She has written 11 research articles and presented several communications in international meetings. The PhD period included a three-month stay (September to November 2017) in the Analytical Chemistry Department of the University of Barcelona (Spain), under the supervision of Prof Martí Rosés, working in the field of Hydrophilic Interaction Liquid Chromatography (HILIC).

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Sugars determination by UPLC-MS/MS and relation with acrylamide formation in bread

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Statement of the Problem: Acrylamide is a carcinogenic contaminant produced during food processing at high temperatures. This contaminant is a Maillard Reaction product that results from a reaction between asparagine and reducing sugars. Therefore, the determination of sugar profile is an important form to understand the formation of acrylamide in foods, such as bread, potatoes or coffee. In the particular case of bread, it is important to understand the effect of fermentation on the formation of acrylamide. Until now, several techniques have been developed to determine saccharides in food, GC-MS, HPLC-DAD, HPLC-FLD, HPLC-ELSD. However, these methods have disadvantages, such as low selectivity and some methods require derivatization. Therefore, the aim of the present work was to develop a method to analyze the mono and disaccharides by UPLC-MS/MS in flour and bread dough.

Methodology & Theoretical Orientation: Six flours (oat, rye, wheat flours) and four bread dough were selected. For the detection and quantification of sugars ultra-performance liquid chromatographic coupled to a mass detector (UPLC-MS/MS) was used. To identify sugars and optimize the parameters of the detector, direct injections of the sugars under study (sucrose, glucose, fructose, maltose, and mannose) was carried out. The following chromatographic conditions were then tested: mobile phase A with acetonitrile/water (10:90) with 0.1% ammonium hydroxide and mobile phase B with acetonitrile/water with 0.1% ammonium hydroxide. The method has a flow of 0.2 ml/min with a 32 minutes gradient: 0-30 min, 100-70% B; 30-30.1 min, 70-60% B; 30.1-31 min, 60-80% B; 31-32 min, 80-100% B.

Result: With this method, it was observed that there are no matrix interferences in the chromatogram (Figure 1), concluding that this method is suitable for sugar analysis in flour and bread dough.

Conclusion & Significance: We developed a simple and rapid method for simultaneous determination of sugars in flour and bread dough to understand the formation of acrylamide in bread

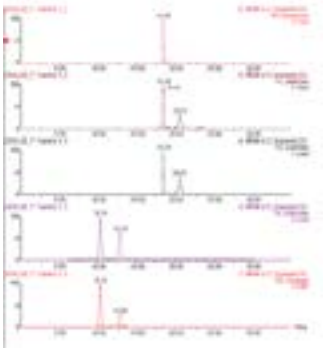

Figure: Chromatogram of sugar profile in flours

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5. Castanheira I, Saraiva M, Rego A and Ollilainen V (2016) EuroFIR guidelines for assessment of methods of analysis: GAMA. Journal of Food Chemistry 193:82-89.

Biography

Susana Jesus is a PhD student in the Chemistry Engineering at Instituto Superior Técnico. She is a Researcher in the MISAGE project-mitigation strategies of acrylamide and advanced glycation end-products in bread. The aim of this project is to mitigate acrylamide in bread through the application of extracts of spices based on antioxidant profile. She has experience in acrylamide determination by UPLC-MS/MS and in simultaneous quantification of sugars with UPLC-MS/MS in food products.

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Lucia Váhovská et al., Biochem Pharmacol (Los Angel) 2018, Volume 7
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HPLC determination of naproxen from mesoporous silica

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[2]Pavol Jozef Šafárik University, Slovakia

Over the last decades, the development of controlled drug delivery systems has increased rapidly. However, recently, there has been growing interest in the use of mesoporous materials as controlled drug delivery matrixes because they have several attractive features, such as stable uniform mesoporous structures, high surface areas, tunable pore sizes with narrow distributions, and well-defined surface properties. The delivery of different drugs using the mesoporous silica materials was investigated, up to now. Our attention has been focused on the study of naproxen release from drug delivery system, based on ordered hexagonal mesoporous silica SBA15. Naproxen, a non-steroidal anti-inflammatory drug (NSAID) derived from propionic acid, is widely used to moderate pain relief in the treatment of many diseases. NSAIDs, including naproxen, are commonly employed to reduce ongoing inflammation, pain and fever, because they are able to block the cyclooxygenase (Cox) enzymes that both produce prostaglandins; these classes of compounds have several important functions, such as the promotion of inflammation, pain and fever. In this work, the released amount of naproxen from SBA15 was monitored in selected time intervals by reversed-phase high-performance liquid chromatography (RP-HPLC). A Dionex Ultimate 3000RS system equipped with a diode array detector (DAD) and programmable Chromeleon Chromatography Data System, Version 7.2, was used for analysis. The mixture of acetonitrile and water (55:45, v/v) adjusted with ortho-phosphoric acid to pH 3 was selected as the best mobile phase. The naproxen was monitored by UV detection at 229 nm.

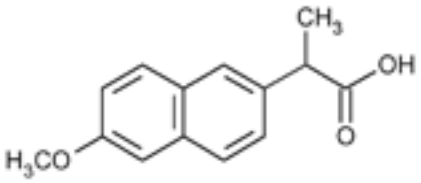

Figure: Structural formula of naproxen

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4. Vitushkina S, Teslenko M, Váhovská L, Findoráková L, Vilková M and Potočňák I (2017) Low-dimensional compounds containing cyanido groups. Part XXXI. First simultaneous nucleophilic addition of water and ethanol to dicyanonitrosomethanide anions in the presence of Co (II). Inorg. Chim. Acta 456:49-54.
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Biography

Lucia Váhovská is a Researcher in the Department of Chemistry, Biochemistry and Biophysics at the University of Veterinary Medicine and Pharmacy in Košice. She has completed her PhD in Inorganic Chemistry at University of Pavol Jozef Šafárik in Košice in 2014. Her academic training and work experience include the preparation of some transition metal complexes with subsequent physico-chemical characterization using infrared spectroscopy, UV-VIS spectroscopy, elemental analysis, RTG structure analysis, magnetic measurement. At present, she participates in a project with the aim to study selected drug releasing (naproxen, 5-fluorouracile) from mesoporous (modified) silica SBA15 using HPLC method.

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Quantification of ethylene diamine tetra acetic acid in an incinerated ion-exchange resin matrix by solid-liquid phase extraction and RP-HPLC-UV analysis

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The University of Manchester, UK

Ethylene diamine tetra acetic acid (EDTA) is found in decontamination agents used throughout the nuclear industry, therefore, is often found in repository consigned wastes. The Low-Level Waste Repository (LLWR) is the UK's Centre for the disposal of low-level radioactive waste. LLWR maintain strict limits of acceptance on EDTA because, when present in the waste, the ligand potentially solubilizes otherwise surface-bound radionuclides, making them more susceptible to groundwater transportation into the wider geo/biosphere. A significant quantity of EDTA and radioactively-contaminated ion-exchange resin sourced from decontamination operations during nuclear submarine maintenance is in radioactive decay-storage in UK Naval dockyards. Before the material can be accepted for disposal at LLWR, it must be thermally conditioned to remove EDTA; and then analyzed to confirm EDTA destruction. A simple method has been developed for the extraction and quantification of EDTA from an incinerated ion-exchange resin matrix using reversed-phase ion-pair high-performance liquid chromatography (HPLC) with ultraviolet detection. EDTA is extracted directly into an aqueous Fe (III) solution to undergo complexation, separated on a monolithic silica column (Chromolith® HighResolution, Merck) and detected at 258 nm. The linearity of the response is high (R2; 0.9999) and the limit of detection/quantification for the method has been determined to be 0.23/0.62 mg/kg, respectively. From the standard addition method on four samples of incinerated resin containing 6% EDTA prior to treatment, high recoveries were obtained (mean value; 78.3±3.34 %), with reasonably high intra- and inter-day repeatability (RSD; 1.42–12.4%). Absorption peaks at similar retention times were observed and, as they do not occur for the resin incinerated without EDTA, are attributed to ferric complexes of EDTA thermal degradation products. Interfering peaks were resolved by applying a least squares fit to the data.

Table

Added Standard (mg/kg)	Recovery (%)	Precision (%RSD)	
		Intra-day	Inter-day
1000	81.5 ± 1.64	1.56	2.01
100	79.8 ± 3.07	2.56	3.85
10	78.0 ± 1.11	1.51	1.42
1	73.8 ± 9.14	9.48	12.4
0	0	0	0

Table: EDTA recoveries from the incinerated ion-exchange resin and the repeatability of the procedure - calculated from four trial runs of the standard addition method over two separate days

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3. Kemmei T, Kodama S, Yamamoto A, Inoue Y and Hayakawa K (2013) Determination of ethylene diamine tetra acetic acid in foods by reversed-phase high-performance liquid chromatography. Food Chemistry 138(2-3):866-869.
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5. Chiumiento F, D'Aloise A, Marchegiani F and Melai V (2015) Determination of EDTA in feed and premix formulations by HPLC-DAD. Food Chemistry 175:452-456.

Biography

James A O'Hanlon is pursuing his PhD at University of Manchester, School of Chemistry, in the group of Prof Melissa A Denecke. He has an Industrial Cooperative Awards in Science and Technology, studentship specially funded by the EPSRC (Engineering and Physical Sciences Research Council) to promote mutually beneficial research collaboration between academic and partner organizations.

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Aysel Berkkan et al., Biochem Pharmacol (Los Angel) 2018, Volume 7
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Determination of fatty acids by head space single drop microextraction gas chromatography (HS-SDME-GC)

Aysel Berkkan and **Aslihan Ekici**
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Statement of the Problem: There are three major aspects in analytical measurements; first, to develop microanalytical methods. Second to perform rapid measurements on small sample volumes. Third, green analytical methodologies. Single Drop Microextraction (SDME) have special value in sample cleanup, rapid extraction and large enrichment factor. So, head space-single drop microextraction (HS-SDME) gas chromatography coupled to flame ionization detector (HS-SDME-GC-FID) was used to determine the fatty acids.

Methodology & Theoretical Orientation: Fatty acids in oil samples were derivatized to improve volatility with esterification reaction. Methylation was carried out by trans-esterification with methanolic potassium hydroxide in order to convert the polar carboxylated groups to less polar methyl ester derivatives. Then, microextraction step was applied in 10 mL head space vial by using the GC microsyringe with a bevel tip that holds the drop. Extraction solvent (sodium dodecyl sulphate: 1-butanol, 1:3), concentration of ionic strength adjustment solution (NaCl), temperature (45°C), and extraction time (35 min) that affect the yield were optimized. Agilent Technologies, Inc. HP-5 column (30mx 0.320mm ID, 0.25 μm film thickness) was used in GC. Inlet and detector temperature were 270°C. Oven temperature program was as follow; 80°C (2.0 min), 4°C/min ramp to 210°C (5 min), 15°C/min ramp to 300°C (5.0 min), helium was used as a carrier gas (1 mL/min flow rate). Injection sample volume was 1 μL with 100:1 split ratio.

Findings: Chromatogram of fatty acids' methyl esters in olive oil was shown in Figure.

Conclusion & Significance: The identification of linoleic acid, and linolenic acid's metyl esters in olive oil were performed by comparing their retention times with the standarts by HS-SDME-GC-FID. The amount of acids was expressed as a percentage (w/w) of all fatty acids' esters detected, linoleic acid (3%), and linolenic acid's (16%).

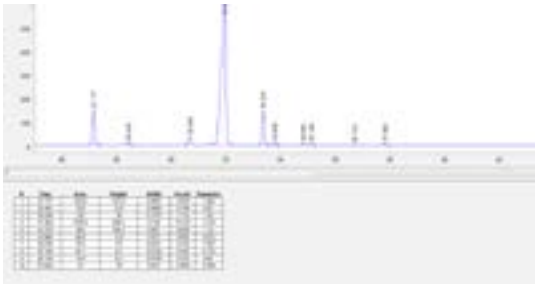

Fig. HS-SDME-GC-FID chromatogram of fatty acids' metyl esters of olive oil

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Biography

Aysel Berkkan has been an Associated Professor at Gazi University, Faculty of Pharmacy, Division of Analytical Chemistry in Turkey since 2014. She received her PhD. degree in 2004 at Gazi University, Faculty of Pharmacy, Division of Analytical Chemistry about Hydride Generation Atomic Absorption Spectrometry with Professor Nusret Ertas. She has been an Assistant Director in the Institude of Medical Sciences at Gazi University in 2016. Dr Berkkan's reseachs involve Hydride Generation Atomic Absorption Spectroscopy, Inductively Couple Plasma- Mass Spectrometry, and Head Space Gas Chromatography.

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Veronica spicata L. - HPLC Profile of Phenolic Compounds in Hydrolates

Nada Bezić[1], Marija Nazlić[1], Ivica Ljubenkov[1], Ivana Mitar[1], Dario Kremer[2], Ivana Anđelić[1], Nenad Vuletić[1] and **Valerija Dunkić[1]**
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[2]University of Zagreb, Croatia

Genus *Veronica L.* (Plantaginaceae) is divided into 13 subgenuses and 270 species, of whom 40 are widespread in Croatia [1]. Samples of the investigated *Veronica spicata L.* were collected in two localities, mountains Dinara and Velebit. They are cosmopolitan and ecologically diverse species spread on a variety of habitats from aquatic, marshy and forest to rock, rock cracks, fields and ruderal habitats [2]. Some of the species from the genus *Veronica* have been used in traditional medicine. Literature reviews showed that the most investigated secondary metabolites for *Veronica* genus include iridoid glucosides, phenylethanoids and flavonoid glycosides [3], so this is the first study of the chemical composition of the essential waters - hydrolates from this species. Hydrolates are aqueous products of hydrodistillation from aerial parts of plant. Aerial parts of the two samples of *V. spicata* were air dried and subjected to hydrodistillation in Clevenger type apparatus. 2mL of pentane and 40 mL of water was added to the inner part of the graduated tube at each distillation. At the end of the process of distillation the organic and water layer were separated and refrigerated until the analyses [4]. The phenolic compounds from hydrolates have been analyzed by Perkin Elmer HPLC system (Waltham, Massachusetts, USA) using the C18 column (Ultra-Aqueous C-18, 250 x 4.6 mm, 5 Å) (Restek, USA). Components 3,4-dihydroxybenzoic acid and vanillin were identified, with the following values. Component in the Sample 1 contained 3.47 mg/L of 3,4-dihydroxybenzoic acid and the Sample 2 contained 6.12 mg/L. Component vanillin in the Sample 1 is represented with 0.12 mg/L and in the Sample 2 with 0.76 mg/L. Our previous studies have included research of volatile components in the composition of the essential oil of *V. spicata* analyzed by GC/MS [4]. Du and Jin found vanillic acid in the species *Veronica* peregrine [5]. The present study gives additional knowledge about volatile compounds in the hydrolates of the genus *Veronica*.

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Biography

Nada Bezić has more than 45 papers published in international scientific journals in the field of virology, anatomy and physiology of xerophytes and their implementation in phytotherapy. Plant species that are the subject of research are spread along Mediterranean part of Croatia. Laboratory researches, apart from exploring plant structures, are focused on the isolation of plant compounds and their chemical characterization, as well as the determination of their biological activity. In the recent years our research was focusing on investigation of the antiviral activity of essential oils. As a professor at the University of Split, prof.dr.sc. Nada Bezić holds lectures and is lead mentor to students at the undergraduate, graduate and postgraduate level.

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Madhuri Singhal, Biochem Pharmacol (Los Angel) 2018, Volume 7
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Quantitative estimation of ß sitosterol from plant *Terminalia bellirica*

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Terminalia bellerica, known as bahera or beleric or bastard myrobalan, common on plains and lower hills in Southeast Asia, where it is also grown as an avenue tree. The leaves are about 15 cm long and crowded toward the ends of the branches. It is considered a good fodder for cattle. *Terminalia bellirica* seeds have an oil content of 40%, whose fatty-acid methyl ester meets all of the major biodiesel requirements in the USA (ASTM D 6751-02, ASTM PS 121-99). In traditional Indian Ayurvedic medicine, Beleric is known as "*Bibhitaki*" (Marathi: "Behada or Bhenda") (*Terminalia bellirica*). Its fruit is used in the popular Indian herbal rasayana treatment triphala. In Sanskrit it is called *vibhīdaka*. The fruit contains anthraquinones and tannins, It is anthelmintic, astringent (especially when ripe), digestive, tonic and laxative (especially when unripe) The fruit is used internally principally in the treatment of digestive and respiratory problems. In Indian herbal medicine the ripe fruit is used in cases of diarrhoea and indigestion, whilst the unripe fruit is used as a laxative in cases of chronic constipation. This paper contains quantitative analysis of ß-sitosterol in various part of *Terminalia bellarica* by using HPTLC method.

Biography

Madhuri Singhal has completed her PhD at Dr Hari Singh Gaur University, Sagar and Postdoctoral work at Allahabad University, Allahabad. Her research area is the role of natural products from medicinal plants in drug discovery and development. She has presented research papers in international conferences in Australia in 2005 and was invited as Visiting Academic in 2006 at Australian University. She has presented papers in USA in 2009 and 2015. In 2010, she has presented research paper at Ubon Ratchathani University, Thailand. In 2011, she has presented paper in Hong Kong. She is an Editorial Board Member of an international research journal. She has published more than 30 research papers. At present, she is a Professor of Chemistry at Government Motilal Vigyan Mahavidyalaya, Bhopal.

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Nada S Ayish et al., Biochem Pharmacol (Los Angel) 2018, Volume 7
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Greenness assessment as per Eco-scale and AMVI matrices for the chromatographic assay of drugs in a semisolid dosage form and in tissues

Nada S Ayish, Ahmed S Saad, Mohamed R El-Ghobashy, and **Badr A El-Zeany**
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A simple green sample preparation procedure and RP-HPLC method were developed and validated for the determination and quantification of drugs in semisolid dosage form as well as in rat rectal tissues. Sample cleanup was performed using solid phase extraction on strata-X prefilled (100 mg, particle size 33µ) 3 ml cartridges, conditions were optimized to obtain maximum recoveries and minimal baseline noise. Separation was carried out on Xselect C18 column (250X4.6 mm id, 5 µm particle size), mobile phase consisting of ethanol: 0.01% aqueous sodium carbonate solution (75:25 by volume) at a flow rate of 1 ml/min and UV detection at 220 nm. The method was validated as per the ICH guidelines. Greenness of the method was assessed throughout the whole procedure of sample preparation, separation and quantification using different matrices and was found to be environmentally friendly as it was also manifested by its greenness profile.

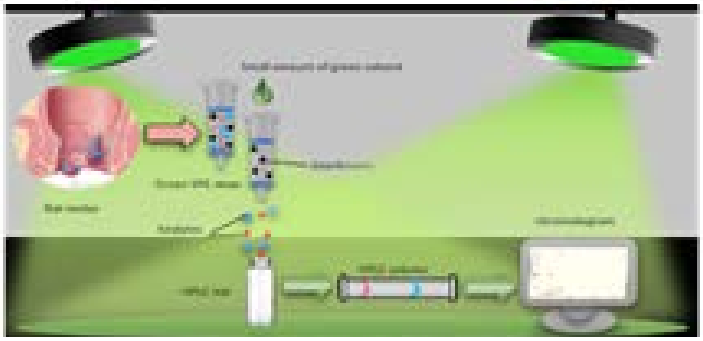

Figure: Graphical abstract representing the chromatographic separation of both drugs from tissue.

Biography

Nada S Ayish a Teaching Assistant in Analytical Chemistry Department, I began my work in research field couple of years ago, in this current work I tried to find a simple rapid and precise method for the determination of these drugs in pharmaceutical formulation as well as in tissues.

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Areeg Z Alkarali et al., Biochem Pharmacol (Los Angel) 2018, Volume 7
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Development of HPLC method for simultaneous determination of four steroid hormones in different matrices

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Since animal products are a major source of human exposure to steroids, the extensive use of these hormones resulted in a critical request for accurate, sensitive, simple and applicable methods for the determination of these steroid hormones in pure form and in different matrices as hen eggs, chicken liver and tilapia farming pond water. The aim of this work is to develop a reliable liquid chromatography coupled with mass spectrometric method for the determination of selected steroid hormones in complicated matrices as hen egg, chicken liver and tilapia farming pond water. Using solid phase and liquid liquid extraction methods for sample preparation, in the present study LCMS/MS method was demonstrated for the simultaneous separation and quantification of four steroid hormones ethinylestradiol, 17 alpha methyl testosterone, testosterone and progesterone. Using mobile phase of methanol and 0.1% formic in ratio (70:30) at different m/z ratios, the method validation was carried out on each hormone showing: linearity for ethinylestradiol 0.5 µg/ml to 30 µg/ml r^2=0.9997, 17α-methyl testosterone 0.5 µg/ml-20 µg/ml r^2=0.9999, Testosterone 0.5 µg/ml-20 µg/ml r^2=0.9999 and progesterone 0.5 µg/ml-20 µg/ml r^2=0.9999. LOD and LOQ values of ethinylestradiol, 17 α methyl testosterone and progesterone respectively (0.7 and 2.12), (0.23 and 0.69), (0.35 and 1.08) and (0.36 and 1.11). The method was validated using the ICH guidelines and successfully applied on egg, chicken liver and tilapia pond fresh water samples from Egypt.

Biography

Areeg Z Alkarali has completed her Bachelor's degree in Pharmaceutical Sciences at Misr International University in 2012 and Master's degree in Pharmaceutical Analytical Chemistry at Suez Canal University in 2018.

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e-Poster

Reyhaneh Sahba, Biochem Pharmacol (Los Angel) 2018, Volume 7
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Application of HPLC techniques in pesticide

Reyhaneh Sahba
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Pesticides of different chemical classes are widely used in agriculture and public health worldwide. As we can see, the amount of pesticides which complicates the task of quality controls, determination of active ingredients (a.i.) was conducted according to official CIPAC, FAO and original manufacturer. The liquid chromatography system is used in the laboratory of PPO for the QC of imported and produced pesticides to the active ingredients (a.i.) content. In this paper, a different approach to the original method of analysis and QC of the reference or the main manufacturer was proposed because of depending on the device model, the purity of the solvents, the length and type of the column. The author has proposed a method for imidacloprid (IMD), SC 35% (insecticide) which has been registered in Iran. A report on the comparison of Bayer's analysis method with CIPAC reference was suggested for determining the a.i. content. The St preparation is used to weight (~0.1 mg) into a volumetric flask IMD St by Dr Ehrenstorfer /138261-41-3/99%. Dissolve in approx. 30 ml of CH_3CN, place the flask in an ultrasonic bath for 15 mins. Make up the flask with water to just below the calibration mark and mix. To prepare the pesticide sample, a sample was transferred to a volumetric flask containing IMD. Continue to work like a ST method. The condition and HPLC system are as follows: reversed-phase method with ODS-3, 5 µm, 250x4.6 mm (i.d) using a mobile phase consisting of acetonitrile/water, HPLC grade (v:v/2:8) at a flow rate of 2 mL/min and UV detection at 260 nm was used. Chromatograms obtained according to Bayer and our method is shown respectively. HPLC capability can be used to design a new optimal method. This method can be used for other formulations of IMD, such as EC.

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Biography

Reyhaneh Sahba has completed her PhD in Chemistry and she works as an expert in the Pesticide Reference Laboratory at Plant Protection Organization of Iran for 11 years. Her work is to execute FAO and WHO orders regarding the quality and health of pesticides in the country.

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Diagnostic accuracy of cannabinoid testing by liquid chromatography-tandem mass spectrometry in human hair

Alveena Younas, Asif Nawaz, Ayesha Hafeez, Aamir Ijaz and **Naveed Asif**
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Objective: To determine the diagnostic accuracy of cannabinoids testing by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS) in human hair and to compare it with urine for detection of cannabis use in civil heavy vehicle drivers.

Materials & Methods: This diagnostic accuracy (validation) study was carried out at Department of Forensic Medical Sciences Laboratory (FMSL), Forensic Toxicology Section, Armed Forces Institute of Pathology Rawalpindi, Pakistan. Hair and urine samples of about 151 civil heavy vehicle drivers were collected from various urban and rural areas of Punjab. Sampling technique was non-probability convenient. About 10 ml of urine volume was collected and stored at -20 °C. Hair strands, about the thickness of a pencil shaft, were collected from the posterior vertex of scalp. It was cut as close to the root as possible, and kept at room temperature till further analysis Separation of compounds was done on Agilent Poroshell 120 EC-C18 column (2.1 x 7.5mm.7 micron) and analyzed on a 6460 Triple Quadrapole LC-MS along with software Mass hunter ©.

Results: All the 151-male civil heavy vehicle drivers, who were included in the study, were categorized into three main groups. There were 69(71.5%) truck drivers, 43(28.5 %) were 20-wheeler drivers; whereas bus drivers were 39(25. 8%).Mean age was 36±10.82 years. Subjects were stratified according to the age into four main groups: 20-25 y: 28(18.5%); 26-40 y: 73(48.3%), 41-60 y: 47(31.1%) and >60 y:3(2%). Paired t test was applied to check significance of study at 95% confidence interval which was significant at $p<0.05$($p=0.00$).Various parameters of diagnostic accuracy in hair and urine samples were: sensitivity (97% and 77%), specificity (92% and 93%), positive likelihood ratio (13% and 12%), negative likelihood ratio (0.04% and 0.24%), positive predictive value (89% and 83%), negative predictive value (98% and 91%) respectively. Overall diagnostic accuracy of cannabinoids in hair was 94.04% while in urine it was 88.67%. Receiving Operating Characteristics (ROC) curve was plotted which showed area under curve of 0.967 and 0.793 for hair and urine respectively, therefore signifying a better diagnostic accuracy of hair as compared to urine for cannabis detection.

Conclusion: This study highlights the importance of hair as an alternative biological matrix due to its good diagnostic yield, easy non -invasive specimen collection and distinctive potential of analyte stability, as well as wider period of detection as compared to urine.

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Administration of 1-deoxynojirimycin attenuates hypothalamic endoplasmic reticulum stress and regulates food intake and body weight in mice with high-fat diet-induced obesity

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[2]Sejong University, Republic of Korea
[3]Catholic University, Republic of Korea

The α-glucosidase inhibitor, 1-deoxynojirimycin (DNJ), is widely used for its antiobesity and antidiabetic effects. Researchers have demonstrated that DNJ regulates body weight by increasing adiponectin levels, which affects energy intake and prevents diet-induced obesity. However, the mechanism by which centrally administered DNJ exerts anorexigenic effects has not been studied until now. We investigated the effect of DNJ in the hypothalamus of mice with high-fat diet-induced obesity. Results showed that intracerebroventricular (ICV) administration of DNJ reduced hypothalamic ER stress, which activated the leptin-induced Janus-activated kinase 2 (JAK2)/signal transducers and activators of transcription 3 (STAT3) signaling pathway to cause appetite suppression. We conclude that DNJ may reduce obesity by moderating feeding behavior and ER stress in the hypothalamic portion of the central nervous system (CNS).

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Optimization of a HPLC method for the analysis of related substances using DoE integrated with the steepest ascent method and Monte Carlo simulation

Gautam Samanta, Trupti Tol, Harshad Tawde, Savita Gorad, Avinash Jagdale, Amol Kulkarni, Avinash Kasbale and **Anita Desai**
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The analytical method for the determination of related substances for formulation of tablets was optimized using quality by design approach. The selectivity issue encountered during oxidative degradation was a stumbling block, with a few poorly resolved degradant peaks (each greater than 1.0%) eluting very close to the main peak and the known impurities. Selectivity (resolution) was considered as a critical quality attribute. Buffer pH, column oven temperature, gradient slope and flow rate were the critical method variables studied through design of experiments. Discovery of an unknown impurity (named as impurity D, about 1.0%) was the key finding from the DoE study. Resolution between impurity D and the main peak and the resolution between the main peak and another impurity, impurity E, were very critical and highly sensitive to change in buffer pH. Moreover, variation in the buffer pH had opposite impact on these two responses. The peaks for API and impurity E were resolved at pH 3.0 and exhibited higher sensitivity towards pH hence the pH value was fixed to 3.0. To improve the separation between impurity D and API, column oven temperature was explored using the method of steepest ascent. Experiments were performed at different temperatures along the path of rapid increase in response, and finally at 45°C, both the critical pairs were well resolved. To achieve the global optima, a response surface methodology was employed. Finally, the optimum condition chosen was pH 3.0, column oven temperature 44°C, % MP. B 45% and flow rate 1.0 mLmin-1. Establishment of design space was complimented by accomplishment of a robust zone through Monte Carlo simulation and capability analysis. An analytical control strategy was designed to ensure that the method repeatedly meets its acceptance criteria. The QbD approach facilitated systematic optimisation of the method, despite various challenges and complications.

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Therapeutic equivalence of generic product versus reference product of ivabradine in patients with chronic heart failure: A crossover study

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Background: Generic substitution of brand ivabradine prescriptions can reduce drug expenditures and improve adherence. However, the distrust of generic medicines by practitioners and patients due to doubts regarding their quality and fear of counterfeiting compromise the acceptance of this practice.

Aim: To compare the therapeutic equivalence of brand product versus generic product of ivabradine in adult patients with chronic heart failure with reduced ejection fraction (≤40%) heart failure with reduced ejection fraction (HFrEF).

Methodology: Thirty-two Egyptian patients with HFrEF were treated with branded ivabradine (Procoralan©) and generic (Bradipect©) during 24 (2x12) weeks. Primary outcomes were resting heart rate (HR), NYHA FC, quality of life (QoL) using Minnesota Living with Heart Failure (MLWHF) and EF. Secondary outcomes were the number of hospitalizations for worsening HFrEF and adverse effects. The washout period was not allowed.

Findings: At the 12th week, the reduction in HR was comparable in the two groups (90.13±7.11 to 69±11.41 vs. 96.13±17.58 to 67.31±8.68 bpm in brand and generic groups, respectively). Also, the increase in EF was comparable in the two groups (27.44±4.59 to 33.38±5.62 vs. 32±5.96 to 39.31±8.95 in brand and generic groups, respectively). The improvement in NYHA FC was comparable in both groups (87.5% in brand group vs. 93.8% in generic group). The mean value of the QOL improved from 31.63±15.8 to 19.6±14.7 vs. 35.68±17.63 to 22.9±15.1 for the brand and generic groups, respectively. Similarly, at the end of 24 weeks, no significant changes were observed from data observed at 12th week regarding HR, EF, QoL and NYHA FC. Only minor side effects, mainly phosphenes, and a comparable number of hospitalizations were observed in both groups.

Conclusion: The study revealed no statistically significant differences in the therapeutic effect and safety between generic and branded ivabradine. We assume that practitioners can safely interchange between them for economic reasons.

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Enantiomeric separation of fluoxetine on C18 column using DFDNB based chiral derivatizing reagent having L-amino acids as chiral auxiliaries

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Optically pure amino acids L-Val, L-Phe, L-Leu and S-methyl-L-Cysteine were used to synthesize chiral derivatizing reagents FDNP-L-Val, FDNP-L-Phe, FDNP-L-Leu and FDNP-SMLC. These reagents were characterized using UV, IR, CHN, and 1H NMR. These all are containing fluoro dinitro benzene as the chromophore and hence are of good molar absorptivity. The synthesis of diastereomers of fluoxetine was carried out by the reaction of these chiral derivatizing reagents with fluoxetine under microwave irradiation for 60s at 75% (of 800W) and also by stirring for 45 min at 50˚C. The diastereomers were enantioseparated by reversed-phase high-performance liquid chromatography on a C18 column with detection at 340 nm using gradient elution with mobile phases containing aq TFA (0.1%)-MeCN in different compositions. The conditions of derivatization and chromatographic separation were optimized. The method was validated for accuracy, precision, limit of detection and limit of quantification.

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Development of FPSE-HPLC-UV method for analysis of phenyltin compounds in environmental and canned food samples

Heena Rekhi
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This paper narrates a novel fabric phase sorptive extraction-high performance liquid chromatography-ultra visible detection (FPSE-HPLC-UV) method for the simultaneous extraction and analysis of four phenyltin derivatives that include triphenyltin hydroxide, triphenyltin acetate, triphenyltin chloride and tetraphenyltin in environmental (agricultural waste water and municipal waste water) and canned food sample. The selected analytes were well resolved by waters nova pack C18 column (3.9x150 mm, 4 µm) in isocratic elution mode within 15 minutes. The new microextraction media has been analytically evaluated using phenyltin derivatives as model compounds. The factors affecting the extraction efficiency of FPSE have been probed and the optimized extraction conditions have been determined. Under these optimum conditions, the limits of detection (LODs) for sol-gel C18 coated FPSE media in combination with HPLC-UV for the analysis of the phenyltin derivatives were in the range of 10-100 ng/mL with precision (relative standard deviation) at 10 ng/mL concentration with good absolute recoveries and less relative standard deviation. To the best of our knowledge, this is the pioneer FPSE extraction procedure applied on environmental and canned food sample for the simultaneous determination of phenyltin derivatives and could be mimic as a rapid and robust green analytical tool.

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Optimization of spherical and cylindrical ion traps

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Amirkabir University of Technology, Iran

Statement of the Problem: Quadrupole ion traps with spherical as well as cylindrical geometries are designed and analyzed. It is worth noting that for a traditional ion trap, the so-called a Paul trap, higher order electric multipole components inside the trap are appeared. These components are attributed to the truncation of the hyperbolic-shaped electrodes of a Paul trap. As a consequence, the electric multipole components higher than an electric quadrupole one have nonlinear effects on the equations of motion for an ion confined in a Paul trap. This nonlinearity causes an anomalous effect on the operation of an ion trap.

Methodology & Theoretical Orientation: To overcome this problem, the Laplace equation is solved for the electric potentials inside the traps with spherical and cylindrical geometries. Afterwards, an optimization is carried out in order to suppress the contribution of the electric octupole component in the potentials inside the traps.

Findings: It is concluded that a spherical ion trap with the electrode caps having the polar angle of 49 degrees can be considered as a pure quadrupole ion trap whereas for the cylindrical geometry the diameter to height ratio of 1.20 makes it possible to operate very similar to the pure quadrupole ion trap.

Conclusion & Significance: Under these conditions, the optimized traps behave like a practical Paul trap. This claim is confirmed by excellent agreement of three stability regions computed for the optimized traps with those obtained for a Paul trap. In addition, fabrication and miniaturization of the spherical and cylindrical traps is much simpler than a hyperbolic Paul trap.

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Molecular cloning involving AAV-CXCL12 gene

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The American Cancer Society reports that this year there will be an estimated 600,920 deaths due to cancer in the United States. Current cancer research includes the use of biomarkers on the surface of cancer cells to distinguish the cancerous cells from normal body cells. Molecular cloning can enhance these biomarkers. Over the past thirty years, molecular cloning has progressed immensely. From digestion to plasmid insertion, the possibilities are endless. The AAV (adeno associated virus) CXCL12 (C-X-C motif chemokine ligand 12) is a protein coding gene that shows great promise with cloning and plasmid insertion. Our project aims to use this gene to bind tightly to biomarkers on the surface of cancer cells. However, before this optimal binding can occur, it is essential to know more about the AAV CXCL12 gene itself. For this reason, our project includes multiple gel electrophoresis assays, plasmid insertion/digestion assays, and PCR purification. From the results of these assays, the efficacy of AAV CXCL12 to bind to cancer biomarkers will become clear. In particular, the cloning assay for the AAV CXCL12 gene holds great potential, as it is possible to clone extraneous DNA into a different host. If extraneous DNA can be cloned into a different host, then there is the possibility of that DNA binding to a biomarker on a cancer cell.

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Dissolving microneedles for the delivery of therapeutics

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The application of nanotechnology in health sciences has experienced an exponential growth over the last 25 years with special focus on drug delivery systems. Transdermal delivery has recently gained importance for its numerous advantages, which include sustainable release, bypass of the pre-systemic hepatic metabolism and patient compliance. Microneedles are designed to circumvent the skin barrier to enhance transdermal drug delivery. They have been produced in various geometries (cone, cylinder, triangular prism, etc) and materials such as silica, polymers or metals. Microneedles made from sugars or water soluble polymers are dissolvable in the skin and release the drug cargo leaving no sharp waste behind. Moreover, polymer microneedles can incorporate larger drug load than other type of needles such as coated or hydrogel swelling microneedles. These dissolving microneedle arrays have been applied in various pharmacological sectors such as gene therapy, vaccine delivery or drug delivery. However, there is still a need of clinical and pre-clinical research before these devices can be released into the market. An essential challenge is to tailor the microneedles dissolution rate to control the release of therapeutics irrespective of the polymeric material. There is also a need to reduce the risk of skin infections during the insertion as microneedles create small pores on the skin. Our research focuses on the development of plasma polymerized surface engineered microneedles for a controlled dissolution and controlled drug release. This one-step, environmental friendly and substrate independent technique will also ease the industrial manufacture. Furthermore, these surfaces can be functionalized to confer antibacterial properties to our microneedles patches to hinder infections and skin damage.

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Evaluating protective effects of *Asperugo procumbens L* on hepatocellular carcinoma in rats

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Asperugo procumbens L. has been used in Iranian traditional medicine to thetreatment of liver diseases. This study evaluated the protective effects of the aqueous extract of *Asperugo procumbents L*. (AAP) against hepatocellular carcinoma (HCC) induced by diethylnitrosamine (DEN) in male Sprague-Dawley rats. HCC was induced in male rats according to the accepted protocol. Briefly, DEN was used as the initiator and 2- Acetylaminofluorene (2-AAF) as the promoter of hepatocarcinogenesis. Firstly animals fasted 96 hours and then re-fed as mitotic proliferative stimuli. After 24 hours re-feeding, rats were injected a single intraperitoneal (i.p.) dose of DEN (200 mg/kg body weight). Two weeks after DEN injection, rats received 14 daily oral dose of 2-AAF (30 mg/kg) for promoting hepatocarcinogenesis. AAP-treated rats received the extract intragastrically at three dose of 100, 200 and400 mg/kg body weight two weeks before the administration of DEN and continued until 8weeks. A significant decrease in serum markers of liver damage and hepatic carcinoma,including alfa-fetoprotein (AFP), gamma glutamyltranspeptidase (GGT), alaninetransaminase (ALT), and aspartate transaminase (AST) were observed in AAP supplemented animals when compared to DEN-treated rats. In addition, AAP counteracted DEN-induced oxidative stress in rats illustrated by the restoration of reduced glutathione (GSH) and the reduction of malondialdehyde (MDA) levels in the liver. The results of morphological and histopathological staining of rat liver showed that AAP-treated animals have an almost normal histological architecture compared to HCC group. The present study provides evidence that *Asperugo procumbents L*. has apotential chemopreventive effect against liver cancer.

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Investigating targets for intellectual disability in autism

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Fragile X syndrome (FSX) is the leading single gene cause of autism and intellectual disability (ID). Neurons express a high density of underdeveloped dendritic spines in FXS humans and animal models. Synaptic plasticity deficits are prevalent throughout the brains of FXS mouse models including the cortex and hippocampus; areas critical for various forms of learning and memory. Moderate to severe learning deficiencies are also characteristic in FXS patients and is paralleled in mouse models. Therefore, FXS is an ideal model in the clinical and laboratory setting to investigate therapies aimed at autism and ID. In FXS mouse models, hyperactive Rac1 has been demonstrated in hippocampus and cortex where dendritic spine abnormalities are a common feature. Herein, we study whether pharmacological regulation of Rac1 might represent a promising treatment for cognitive impairment in autism, using Fragile X syndrome (FXS) as a model. Our results show that in the Fmr1 KO mice (an animal model of FXS) deficits in memory and synaptic plasticity are associated with the presence and mislocalization of Rac1. Interestingly, treatment of Fmr1 KO mice with a specific Rac1 inhibitor improves memory and increases hippocampal LTP. Taken together these observations suggest that Rac1 might contribute to FXS related learning and memory impairments in humans. Importantly, this study proposes that targeting Rac1 in FXS may rescue cognitive impairments. Such a therapy may be translated into broader applications in autism and ID.

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Chromatographic purification of enveloped viruses

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Statement of the Problem: Mumps and measles virus are enveloped, RNA viruses that cause mumps and measles in humans, respectively. Mumps and measles virus particles are used in production of prophylactic vaccines and as gene vectors and oncolytic agents. Application of virus particles as biopharmaceutics requires highest purity to ensure potency and safety of the medicine. Impurities present in crude virus suspensions originate either from host cells (e.g. cellular DNA, exosomes), cultivation medium (e.g. BSA), from processing (e.g. extractables, leachables) or from the virus itself (e.g. aggregates, empty capsids).

Methodology & Theoretical Orientation: Due to the virus delicate macromolecular structure purification process needs to be powerful but gentle. Chromatography is gaining increasing interest in this regard especially due to development of monolithic columns. We have tested three modes of chromatography for purification of mumps and measles virus; ion-exchange, hydrophobic interaction, and affinity chromatography. Recovery of procedures was monitored by cell culture infectivity assay and measurement of total particle concentration using NanoSight. Host cell genomic DNA and proteins were measured using PCR and ELISA, respectively.

Results: Results showed that mumps and measles virus both bind strongly to anion exchange columns, but recovery of infective particles is below 20%. Immunoaffinity chromatography was performed using novel approach of elution with amino acids of high molarity at neutral pH. This approach was found effective for elution of functional virus particles and recoveries around 70% were obtained. Hydrophobic interaction chromatography was also successful with recoveries around 60%. Interestingly, recovery of infective virus particles in hydrophobic interaction chromatography was found to depend on the total-to-infective particle ratio in the starting crude virus suspension.

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The study of the antibacterial effect of aqueous extracts of *Laurus nobilis* leaves using different methods of extraction

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The use of antibiotics is important to combat bacterial diseases. But due to the increasing misuse of antimicrobial drugs that are used in the treatment of infectious disease, multiple resistances have been developed. The effect of aqueous extracts of bay leaf (*Laurus nobilis*) using different extraction methods (maceration and decoction) against *Staphylococcus aureus* and *E.* coli were evaluated *in vitro* using agar-well diffusion method. The results of the study showed non-significant differences between methods of extractions. The study concludes that *in vitro* antibacterial activity may be potentiated against test strains by using other methods of extraction and solvents which may be useful to overcome antimicrobial resistance.

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The determination of the anti-coagulant property of sulfated glycosaminoglycan from the cephalothorax of white leg shrimp (*Penaeus vannamei*) family: Penaeidae

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In this study, the anticoagulant property of sulfated glycosaminoglycan was evaluated using plasma recalcification test. Extraction of the sulfated glycosaminoglycan from the white leg shrimp was performed by defatting the sample with acetone. The deffated sample was treated with 0.4 M sodium sulfate and aluminium disulfate crystal to collect the supernatant. The supernatant was treated with 90% ethanol. The mixture was centrifuged using a refrigerated centrifuge at 8000 rpm for three minutes and the collected precipitate was washed using absolute ethanol. The sulfated glycosaminoglycan was tested using plasma recalcification test. The results in the said test showed that at 30 ug/mL was significant and at 60 ug/mL and 90 ug/mL were very significant. This showed that sulfated glycosaminoglycan from the white leg shrimp exhibited an anticoagulant property.

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Isolation of a trypanocidal sesquiterpene lactone from *Stevia gilliesii* by chromatographic techniques

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Chagas disease is an illness caused by the protozoan *Trypanosoma cruzi* which affects about 6-7 million people worldwide. Sesquiterpene lactones (STL) are a large group of secondary metabolites mostly found among Asteraceae family plants. Sesquiterpene lactones present a wide range of biological activities such as anti-inflammatory, cytotoxic, antiplasmodial and antitrypanosomal, among others. In a previous work, we have evaluated the effect of a group of STLs against T. cruzi epimastigotes and their cytotoxicity against Vero cells. *Eupahakonenin B* (EKB), isolated from *Stevia gilliesii* var. gilliesii Hook. and Arn. (Asteraceae) has shown promising trypanocidal activity and selectivity index (IC50=0.78 μM; CC50=363.72 μM; SI=466). In this work we describe the isolation of EKB by chromatographic methods in order to assess its trypanocidal activity against infective and intracellular forms of T. cruzi. Dried aerial parts of *S. gilliesii* var. gilliesii were extracted twice with dichloromethane. Dichloromethane extract was partitioned between hexane and a water/ethanol mixture and fractionated by column chromatography using silicagel and dichloromethane/ethyl acetate (1:2) as mobile phase. Eluted fractions were analyzed by thin layer chromatography (sp: silica gel 60 F254, mp: Dichloromethane/ethyl acetate 1:2). EKB containing fractions were joined and taken to dryness to obtain the compound as a greenish gum. EKB purity was analyzed by HPLC. The effect of EKB on bloodstream infective form of T. cruzi was assayed by counting remaining living parasites in a Neubauer chamber and the effect of EKB on intracellular forms of T. cruzi was assayed using β-galactosidase transfected parasites as previously described. The sesquiterpene lactone EKB appeared as a blue spot on TLC after spraying the plate with anisaldehyde-sulphuric acid (Figure 2). The STL was purified and isolated by chromatographic techniques (96% purity) and identified by spectroscopic methods (Figure 2). This STL showed activity and selectivity on trypomastigote (IC50=30 μM; SI=11) and amastigote (IC50=29.9 μM; SI=12) forms of T. cruzi. The mechanism of action of EKB and its *in vivo* activity will be evaluated.

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A study on the knowledge, attitude and practice of generic medicines among the doctors in a tertiary care teaching hospital in North-east India

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Prescription drug spending is increasing and out-of-pocket expenses come to 80% of total health-care expenditures. Generic drugs are typically less expensive than brand-name drugs with same therapeutic effect; however, many doctors hold negative views of generics and resist prescribing. The aim of this study was to evaluate the knowledge, attitude and practice of doctors regarding generic medicines and to explore the factors hindering and favoring generic drug prescribing if any. It is a cross-sectional questionnaire-based study in a tertiary-care teaching hospital. All doctors working in the hospital during the study period participated and filled up the structured and pre-validated questionnaires which were then analyzed. Close to three quarters of the participants had good attitude about the efficacy and safety of generic drugs and majority of doctors actively prescribe it but a high number of doctors (72%) were of the view that generics were manufactured with poorer quality but cheaper than brand name drugs. Majority of the respondents believed that their prescribing decision is influenced by a lot of factors. These results suggest that there are significant numbers of concerns about the quality of generics amongst doctors and these negative perceptions are likely to be barriers to a wider acceptance of generics. In order to have a better understanding of generics, the doctor must be well-informed about the generic during their academic career resulting in savings of healthcare budgets.

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Syringic acid, a phenolic compound attenuated arthritis by inhibition of cytokines in complete freund's adjuvant induced arthritis in rats

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Aim & Objective: To evaluate the anti-arthritic potential of syringic acid in Freund's Complete Adjuvant induced arthritis in rats and to study the underlying mechanism.

Methods: Rheumatoid arthritis was induced in male Wistar rats by sub-plantar injection of 0.1 ml of Complete Freund's Adjuvant into right hind paw on day 0. The treatment of syringic acid (25, 50 and 100 mg/kg) and standard drug, indomethacin (1 mg/kg) was started from day 0 and continued up to day 21. The body weight, paw volume, paw thickness and arthritic index were determined on day 0, 3, 7, 10, 14, 18 and 21. On day 22, rats were sacrificed and hematological, biochemical, anti-oxidant parameters, the thymus and spleen indices and cytokine level were estimated. Histopathological examination of the injected paw of the rat was performed.

Results: Syringic acid showed significant ($p<0.05$) reduction in paw volumes at doses 50 mg/kg and 100 mg/kg. Syringic acid showed significant reduction in tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) levels in the serum, while increase in the anti-oxidant and biochemical parameter. The histopathology showed reduced cellular infiltration, synovial line thickening and joint erosion of cartilage.

Conclusion: The restoration of the levels of TNF-α and IL-6 to normal may be contributing to the anti-arthritic potential of syringic acid and could be a promising therapeutic alternative in the treatment of rheumatoid arthritis.

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Determination of o-phthalic acid esters in low alcohol wines by gas chromatography-mass spectrometry coupled with emulsion liquid-phase microextraction preconcentration

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Esters of o-phthalic acid are very dangerous for human health. Their occurrence in wines is connected with the inflow from the plasticized polymer seals, plastic piping, tanks and stoppers. In this study the high sensitive gas chromatographic-mass spectrometric determination of phthalates in low alcoholic beverages (champagne, red and white wine) coupled ultrasound-assisted emulsification-microextraction was developed. As extractants environmentally friendly hydrocarbons - n-heptane and hexane are proposed.The sources of possible systematic errors were investigated: leaking of o-phthalates from chromatographic septum; contamination of phthalate in solvents; influence of macro components of wines (sugar, alcohol and anthocyanin); the hydrolysis of o-phthalates and others. For the first time it is shown that the impact of these factors can lead to an overestimation or underestimation of the actual concentration of impurities by 1-2 orders of magnitude. The methods of accounting or elimination of systematic errors are proposed. Purification of solvents by Rayleigh distillation method allows obtaining samples with impurity content lower than (1-4) ·10-3mgL^{-1}. Containers for sampling and storage of samples to be analyzed should be made of borosilicate glass or quartz. The content of o-phthalates in wines was 0.03 - 1 mgL^{-1}. The largest concentrations are characteristic for diethyl-, di-n-butyl- and di (2-ethylhexyl) phthalates. The limits of detection of esters of o-phthalic acid in low alcohol beverages achieved are at the level of 10-6–10-5 mgL^{-1} and are highly competitive with the best world results. Therelative expanded uncertainty of the determination of toxicants is at the level of 13- 30%.

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