Phd thesis

Analytical examination of human and phytoestrogens

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Introduction

Physiology and health aspects of sexual steroids

The sexual steroids are essential biomolecules for human and animal organisms with strong biological activity at low concentration. They are synthesized mainly in the gonads and in the reticular substance of the adrenal gland. The biosynthetic enzymes are located in the inner membrane of the mitochondria and in the endoplasmatic reticulum. Changes in the amount of the circulating sexual steroids (mainly estrogenes) are the cause of several frequent diseases like breast or prostate cancer, or osteoporosis.

The main difficulties in the analysis of steroids are their high structural similarity and their occurrence at low concentration. Mass spectrometry (MS) seems to be a good approach, but it results in poor sensitivity due to the chemical features of the steroid hormones. This is commonly solved using derivatisation prior to the analysis. During our work we intended to develop a matrix-assisted laser desorption/ionization time-of-flight (MALDI TOF) MS based method to detect underivatised sexual steroids (testosterone, estrone, estradiol, estriol and progesterone).

Occurrence biological importance and photoisomerisation reaction of the phytoestrogene trans-resveratrol

Phytoestrogenes are naturally occurring secondary plant metabolites which are structurally similar to the 17α-estradiol. Plant estrogenes have biological activity in animal organisms too. These, chemically polyphenol type compounds are synthesized by plants in response to different stress factors like UV light or microbial infection, mainly in the epidermal tissues of the leaves and fruits. A commonly studied representative is the resveratrol (3, 4', 5 trihidroxistilbene), which was first isolated from the grape (*Vitis vinifera*) leave. Resveratrol is found in a considerable amount in red wines too, which alcoholic environment is thought to have a positive effect on the imbibition and digestibility of polyphenols. Resveratrol is present in free or conjugated forms, the latter is called piceid. Resveratrol also shows trans-/cis-photoisomerization, the reaction is catalyzed by UV light. The dietetic importance of trans-resveratrol lies in its biological activity. The molecule is a strong anti-oxidant, has a free-radical scavenging nature, and further it can bind to the known estrogene receptors. Because of that, trans-resveratrol can decrease the harmful effects of free radicals, decrease the risk of

coronary heart disease and can compensate the consequences of estrogene deficient states (osteoporosis). Since because of the above discussed reasons wines are excellent sources of polyphenols we found the trans-resveratrol and trans-piceid-content of Hungarian wines to be an interesting question. We also wanted to study the photoisomerisation reaction of resveratrol, because the literature data is not consistent in the question of the number of products derived from the photoisomerisation reaction. In some publications we can read about only one observed reaction product (cis-resveratrol), but other authors report two, or even three products.

Aims

During our work we tried to answer the following questions:

1. We wanted to determine the trans-resveratrol and trans-piceid content of Hungarian wines.

2. By comparing the vintage years, the wineries, and the wine types we tried to find the major factors affecting the resveratrol content of wines.

3. We intended to study the photoisomerisation reaction of trans-resveratrol to find out whether only cis-resveratrol is the product of this reaction, or do any further compounds arise from the reaction.

4. If there are any new compounds present beside the cis-resveratrol, we aimed to identify them using HPLC, MS and MS/MS methodology.

5. Further, we wanted to develop a MALDI TOF MS method using fullerenes as matrix compounds. Our purpose was to examine sexual hormones in tissues and body fluids with high sensitivity without the use of derivatising reagents.

Materials and Methods

Trans-resveratrol and trans-piceid standards

Trans-resveratrol (99%) and *trans*-piceid was purchased from Sigma-Aldrich (Budapest, Hungary). Ethanol was purchased from Reanal Rt. (Budapest, Hungary), and methanol (HPLC-grade) from Scharlau Chemie S.A. (Barcelona, Spain). All other chemicals were of analytical grade. Freshly distilled water was used in solvents, and for preparation of aqueous solutions.

Sexualsteroid standards

Estrone (1,3,5(10)-estratrien-3-ol-17-one), β-estradiol (3,17β,-dihydroxy-1,3,5(10)-estratrien), estriol (1,3,5(10)-estratrien-3,16α,17β-triol), progesterone (4-pregnene-3,20-dione) and testosterone (17β-Hydroxy-3-oxo-4-androstene) (Sigma-Aldrich Kft., Budapest, Hungary) were used as analytical steroid standards. The reference solutions were prepared by dissolving 0.1 mg of the steroid hormones in 1 ml of methanol (Scharlau Chemie S.A. Barcelona, Spain). Following the complete dissolution 200 µl of MilliQ water was added to the solution.

Fullerene matrix

The C_{70} fullerene (Gold grade) was purchased from Hoechst AG (Frankfurt, Germany). The fullerene matrix was prepared as a saturated solution in toluene; the excess was removed by centrifugation. A thin layer of C_{70} fullerene crystals was formed on the surface of the target plate (MTP 384 target plate ground steel, Bruker Daltonics, Bremen, Germany) by placing the saturated toluene C_{70} solution on the corresponding spots. After the evaporation of toluene 1 µl of each analyte solution was dropped on top of the fullerene crystal layer.

Wine samples

42 different wines were collected from the Hungarian "Villány" and "Eger" wine regions. Wines samples from "Villány" were provided by the Bock and Polgár wineries from the 2003 to the 2007 vintage years. Samples from "Eger" were provided by the Korona winery from 2007 vintage years.

Photoisomerisation reaction of trans-resveratrol

The solid *trans*-resveratrol standard (0.5 mg) was dissolved in 1 ml of ethanol in order to provide complete dissolution. It was than filled up to 10 ml with 15/85 (V/V) ethanol water mixture. Than a median dilution was performed providing a 0.05 mg/ml concentration stock, which was than UV irradiated in darkness with Cole Palmer 9815 lamp (Cole Palmer Instrument Co., Vernon Hills, Il) at 365 nm. 200µl samples were taken out after 10, 30, 60 and 300 minutes and were kept in dark at 4^{o}C until the measurement.

Forensic material

The bone fragments were trimmed free of any residual soft tissues and washed with phosphate buffer saline (PBS) and distillated water to remove contaminants. Bone powder was ground by hand with an agate mortar; till ca. 0.2 mm particle size was achieved. Steroid hormones were extracted from the pulverized bone material as follows: 100 mg of calcificated bone powder was homogenized with 10 ml of dichloromethane (LiChrosolv, Merck KGaA, Darmstadt, Germany) in an ultrasonic bath at 15 minutes. The extract was centrifuged and the supernatant was collected. The supernatants were evaporated to dryness at room temperature and the solid residues were re-dissolved in 10 µL of dichloromethane/methanol/water (7:2:1, v/v/v).

Urine samples

The urine sample was collected from a 29-year-old pregnant individual in the third trimester. Hormone extraction was carried out in the following way. First the sample was centrifuged at 4000 rpm at 4˚C for 20 min. 1 ml of the supernatant was then extracted with 1 ml of dichloromethane three times. The solvent was evaporated under vacuum and the residue was redissolved in 10 µL of acetonitrile/water (9:1) v/v.

HPLC analysis of trans-resveratrol and trans-piceid content of wines

The HPLC system used consisted of a Dionex P680 gradient pump (Dionex Corp., Sunnyvale, CA), a helium degassing system, a Rheodyne 8125 injector valve with a 20µl loop (Rheodyne Europe GmbH, Bensheim, Germany), and a Dionex 340D UV/VIS diode array detector

(Dionex Corp., Sunnyvale, CA). A 250×4.6 mm column has been filled with homemade 6µ particle size C_{18} material. A Chromeleon data management software (Dionex Corp., Sunnyvale, CA) was used to control the equipment and for data evaluation. A multistep gradient method was applied as described previously. The chromatogram was monitored at ambient temperature at 285 and 306 nm, where both compounds have their absorbance maxima. Peaks were identified by retention times and by UV spectrum of the respective compounds in comparison with those of the references.

HPLC-APCI mass spectrometry

The HPLC system was connected to a Bruker HCT Esquire (Bruker Daltonics, Bremen, Germany) MS instrument through a microsplitter valve (Upchurch Scientific, Oak Harbor, WA), the flow rate was 1.5 $cm^3 \times min^{-1}$ with a splitting ratio of 7 over 3. The mass spectrometer equipped with an atmospheric electrospray ionization source was employed for mass detection. The ion source was operated in positive mode. Nitrogen was used as drying gas at 250 ºC, with a flow rate of 5 litre per minute, the pressure of the nebulizer was set at 30 psi. We used the Smart Parameter Setting (SPS) with target masses of 229.2 and 227.2 m/z. The scanning mass to charge range was 50 to 2000 m/z with a scanning speed of 8100 m/z per second. Maximum accumulation time was 200 ms. For the MS-MS analysis, we choose manual MSn for fragmentation of 227.2 and 229.2 precursor ions. For control of the instrument, an Esquire Controll Version 5.3 Build 11, and for data evaluation a Data Analysis Version 3.3 Build 146 was used, both obtained from Bruker Daltonics.

MALDI TOF mass spectrometry

The mass spectrometer used was an Autoflex II TOF/TOF (Bruker Daltonics, Bremen, Germany) operated in reflector for MALDI TOF with an automated mode using FlexControl 2.4 software. An accelerating voltage of 20.0 kV was used for analysis. The instrument uses a 337 nm pulsed nitrogen laser, model MNL-205MC (LTB Lasertechnik Berlin GmbH., Berlin, Germany). External calibration was performed using the monoisotopic quasimolecular and dimer ion peaks of α-cyano-4-hydroxycinnamic acid and 2,5-dihydroxybenzoic acid matrices. Hormone masses were acquired with a range of m/z 200 to m/z 500, each spectrum was produced by accumulating data from 500 consecutive laser shots. The Bruker FlexControl 2.4 software was used for controlling the instrument and the Bruker FlexAnalysis 2.4 software for spectra evaluation.

Results and Discussion

Trans-resveratrol and trans-piceid content of Hungarian wines

In the present work 42 wine samples were analyzed from the 2003-2007 vintage years representing three wineries, and two geographically distinguished regions. *Trans*-resveratrol concentration spanned between 0.4 and 10.4 mg/L while *trans*-piceid between 0.1 and 4.4 mg/L. Remarkably high amounts of *trans*-resveratrol was found in Bock Cabernet Sauvignon (2006) 10.4 mg/L and Bock Merlot (2006) 7.2 mg/L. Wines of the Polgar winery contained in average similar amount though *trans*-resveratrol concentrations under 2 mg/L were not observed. The highest amount of *trans*-resveratrol was 5.1 mg/L found in Polgar Syrah 2005. In the wines collected in "Eger" wine region the concentrations of *trans*-resveratrol were lower, spanning between 0.4 mg/L to 2.5 mg/L with the highest value found in Egri Blau Burger (2007). *Trans*-piceid concentration ranged from 0.1 mg/L to 4.4 mg/L in the analyzed wines. Very high amounts were found in Bock Blaufrankisch (2007) 4.4 mg/L, and "Egri" Blau Burger (2007) 3.7 mg/L. We did not observe significantly lower *trans*-resveratrol concentrations in the more aged wines like Bock royal Cuvee (2003), or Bock Blaufrankisch (2003) showing that the alcoholic solution provides a stabile environment. This is in correlation with literature data. In Bock Cabernet Franc (2003) hoverer we could not detect any of the two compounds but probably this is due to different factors.

The investigation also involved one white and one rose wine. Compared to the red wines lower *trans*-resveratrol and *trans*-piceid were expected due to differences in wine technology. Concentrations of *trans*-resveratrol and *trans*-piceid in the white wine, Bock Capella Cuvee (2003) were 0.7 mg/L and 0.1 mg/L respectively. In the rose wine, Bock Rose Cuvee (2007) similar amounts were detected with 0.5mg/L for both *trans*-resveratrol and *trans*-piceid. In comparison with data found in literature we can conclude that *trans*-resveratrol and *trans*-piceid are found in Hungarian wines in a relatively high amount, especially in the southernmost "Villány" region.

As wines being a good source of trans-resveratrol and trans-piceid, rapid determination of their concentration in foodstuff or wines is of great interest because of dietary purposes. Results of our measurements provide a set of data comparing several varieties of wines, produced in two geographically different regions in Hungary. The highest trans-resveratrol concentrations were found in Merlot, Cabernet Sauvignon and Syrah. It was also noticed that comparing wines within the 2007 vintage year, wines produced in "Villány", located in the

southern, warm climate part of Hungary contain significantly higher amounts of trans-resveratrol and trans-piceid than of those produced in "Eger", where the climate is cooler and more humid. Next to differences in wine variety and wine making technology, climatic factors might influence the polyphenol content of wines.

Photoisomerisation reaction of trans-resveratrol

In the control sample only a single peak was detected, which eluted at 21.9 minutes. According to the retention time, and UV spectrum the peak was identified as trans-resveratrol. After ten minutes of UV irradiation at 365 nm a second peak appeared in the chromatogram, eluting at 23.5 minutes, representing the cis- stereoisomeric form of resveratrol. This second peak was followed by another, low intensity peak at 23.8 minutes. In the samples irradiated for half and one hour the area of this unknown peak grew significantly in correlation with some other reports, that the cis-resveratrol molecule is not the only product originating from the isomerisation reaction. The area of this peak reached it's maximum in the one hour irradiated sample as peak height did not grew significantly after two hours. In the experiment where five hours of UV irradiation was applied, we could not detect any further products. UV-Vis spectra of the three peaks have also been collected, and it was found that the UV-Vis spectrum and absorbance maxima for cis- and trans-resveratrol are the same as described in literature, and for the third peak the absorption maximum at 302.4 nm is very close to the absorbance maximum of trans-resveratrol. The absorption spectrum of the unknown compound was also similar to that of trans-resveratrol, but we observed an additional peak at 249.7 nm. The mass spectrum of trans-resveratrol showed an intense peak at 229.2 m/z, followed by a peak of 230.2 m/z and 231.2 m/z as isotopes of carbon. MS spectrum of cis-resveratrol proved to be exactly the same as the trans- form. The third component was detected at 227.2 m/z. The MS/MS spectrum of trans-resveratrol showed a fragment at 210.9 m/z representing the loss of one hydroxyl group as water, another fragment at 192.9 m/z representing the loss of two water units. The fragments at 134.9 m/z and 119.0 m/z contain the phenolic ring of the molecule with a short carbon chain and a triple bond. A similar fragmentation pattern was found for cis-resveratrol. The fragmentation of the third peak however provided fragments at 209.0 m/z, 191.0 m/z and at 135.0 m/z and 119.0 m/z respectively. Structure identification of the unknown compound The 2 Da difference between 227.2 and 229.2 m/z in the MS spectrum can originate from the loss of two hydrogen atoms suggesting that the unknown substance is an oxidation product. Oxidation might eventuate at

the hydroxyl groups providing a keto- group or at the double bond in the centre of the molecule providing a triple bond. Third possibility is a photocyclisation reaction to a phenanthrene derivative, which is a well known reaction although it is only potential from the cis- form of stilbenes. All three molecules would have the same 227.2 Da molecular mass as quasimolecular ions.

The oxidation of the hydroxyl groups to keto- groups can be precluded as the UV-Vis spectrum of the unknown compound did not notably differ from the spectrum of the trans-resveratrol. The only significant difference is an additional peak at 249.7 nm. The results of the MS/MS experiments also excluded this possibility. The two fragments loosing the water units at 209.0 m/z and 191.0 m/z (2 Da difference) can only be observed if the two hydrogen atoms are missing from the centre of the molecule, as water loss is not possible from the keto-groups. It is also noticeable that the fragments at 135.0 m/z and 119.0 m/z are present in all the three compounds MS/MS spectra. This is due to the formation of a triple bond during the fragmentation of the standard resveratrol molecule.

In literature a phenanthrene derivative from cis-stilbenes is described as a potential product of UV photoisomerisation reaction where the cis-stilbene product undergoes a photocyclization reaction to phenanthrene. To test this possibility an experiment was performed where cis-resveratrol was enriched in a previous isomeration mixture and was irradiated at 312 nm. After 2 hours of irradiation beside of the three already existing components a new component appeared which eluted at 23.53 min not overlapping with the peak of the unknown substance . To ensure that this new peak is the assumed phenanthrene derivative semiempirical quantumchemical calculations were carried out using the Hyperchem software. The peak maxima in the calculated UV-Vis spectrum of the 2,5',7-trihydroxy-phenanthrene proved to have a significant correlation with the maxima of the observed spectrum. It also notably differed from the UV-Vis spectrum of the unknown compound. At this point MS/MS experiments would not provide convincing results as the observed fragmentation pattern is conceivable from both structures.

The mass spectrometric and UV-Vis spectral results indicate that the third unknown compound is a triphenyl-acetylene derivative of trans-resveratrol where the double bond at the centre of the molecule, changes into a triple bond forming 3,4',5-trihydroxidiphenylacetylene (TDPA). For further confirmation of this molecular structure semiempirical quantumchemical calculations were performed using the Hyperchem software. The calculated spectrum of TDPA and the observed spectrum of TDPA (the absorbance maxima) are near to each other (mostly within 1.5 nm) demonstrating the probability of the proposed structure.

MALDI TOF mass spectrometric analysis of sexual steroids

All compounds were analysed in both positive and negative ion modes with different results. The detection of estrogenes was successful in negative ionization mode, while the detection of testosterone and progesterone in positive ion mode. Mass spectra of the five sexual steroids were finally collected in negative ion mode for estrone, β-estradiol and estriol, and in positive ion mode for progesterone and testosterone. The observed phenomenon was explained by the functional groups of the steroid structures. The detection of estrogenes was more successful in the negative ion mode, because they contain more hydroxyl- then keto- groups. At the hydroxyl-groups deprotonation, which accompanies the negative polarity mass spectrometric ionization more easily eventuates. Testosterone and progesterone however contains more keto-groups, so positive ionization and protonation is conceivable. Therefore estrogenes were detected as negatively $[M-H]^-$, and testosterone and progesterone as positively $[M+H]^+$ charged quasimolecular ions. The limit of detection using C_{70} fullerene was 40 pmol, and mass accuracy was 5 ppm. Because of the discussed reasons the urine, and bone samples were only analyzed with C_{70} fullerene.

Analysis of the pregnant urine sample was performed in both negative and positive ionization modes. In urine, steroids are mostly found in a conjugated form to facilitate urinary excretion, but free forms are also present though in a low concentration. In negative ion mode free estriol (the classical steroidal marker for pregnancy) and estradiol sulphate was detected. In positive ionization mode progesterone was observed.

Gender estimation of forensic and anthropological material is commonly done using the tubular bones and the pelvis where sexual dimorphism is best represented. These methods however do not hold up when only fragmented material is available. Therefore in our research we extracted the common sexual steroid hormones from pulverized bone samples and used them as molecular sex markers of forensic remains. The results were confirmed by forensic examination, and DNA analysis.

In our research two forensic cases were analyzed, samples were measured using positive and negative ionization modes too. In negative ion mode we did not observe estrogenes, in positive ion mode however the positively charged quasimolecular ion of testosterone was detected in both samples. The results showed that both remains were male, which was in correlation with the conclusion of the forensic examination.

Summary

During the first part of our work we established the trans-resveratrol and trans-piceid content of Hungarian wines using a validated HPLC assay. We have compared the „Eger" and „Villány" wine regions, and more (2003-2007) vintage years.

- We have found, that in the Hungarian red wines, especially in the ones from „Villány" a remarkable amount of trans-resveratrol is found. This was an average of 3-3.5 mg/l while according to literature this is used to be 0.5 és 2.9 mg/l worldwide.
- Merlot, Syrah, Cabernet Sauvignon, and Pinot Noir wine types contain the most trans-resveratrol.
- We also found, that the good agricultural years are usually accompanied by relatively high trans-resveratrol and trans-piceid content in wines.
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In the second part of our research we studied the photoisomerisation reaction of trans-resveratrol using LC-MS and MS/MS methods.

- It was found, that not only cis-resveratrol, but other products were also formed during the photoisomerisation reaction.
- One of the products is the 2,5',7-trihydroxi-phenantrene, which is the well known product of the cis-reseveratrol photocylisation reaction.
- The other, yet unknown compound was formed from the trans-resveratrol.
- By the results of MS, and MS/MS analysis this molecule was determined as a acetylene derivative (3,4',5-trihydroxi-diphenilacetilene).

The third field of our research was the mass spectrometric analysis of sexual steroids.

- Using fullerenes as matrix compounds we managed to develop a new MALDI TOF MS based method for the analysis of underivatised steroids. We managed to detect the five most common sexual steroids, estrone, estradiol, estriol, progesterone and testosterone.
- Our method is the first case where fullerenes were used as MALDI matrix compounds.
- We successfully detected male and female sexual steroids in pregnant urine and in forensic bone material.

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List of publications:

Articles included in the thesis

1. Determination of products derived from trans-resveratrol UV photoisomerisation by means of HPLC–APCI-MS.
Gergely Montsko, Martin S. Pour Nikfardjam, Zoltan Szabo, Katalin Boddi, Tamas Lorand, Robert Ohmacht, Laszlo Mark.
Journal of Photochemistry and Photobiology A: Chemistry (196) 1: 44-50 (2008) IF:2.36

2. Analysis of nonderivatized steroids by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using C70 fullerene as matrix.
Gergely Montsko, Alexandra Vaczy, Erzsebet Mernyak, Eva Frank, Zalan Kadar,Robert Ohmacht, Janos Wolfling, Laszlo Mark.
Analytical and Bioanalytical Chemistry 395(3): 869-74 (2009) IF: 3.33

3. Trans-resveratrol and trans-piceid content of Hungarian wines.
Gergely Montsko, Robert Ohmacht, Laszlo Mark
Chromatographia 71(1): 121-124 (2010) IF: 1.31

4. Hormone Mass Fingerprinting: Novel possibility for high-throughput molecular sex determination of human skeletal remains by MALDI TOF mass spectrometry.
Gergely Montsko, Gabor Maasz, Zoltan Patonai, Istvan Bajnoczky, Brigitta Osz, Antonia Marcsik, Laszlo Mark
Forensic Science International (In press) IF: 2.01

5. Investigation of phenolic components of Hungarian wines.
Avar Péter.; Pour Nikfardjam Martin; Kunsági-Máté Sándor; **Montskó Gergely;** Szabó Zoltán; Böddi Katalin; Ohmacht Róbert; Márk László
International Journal of Molecular Sciences 8 (10): 1028–1038 (2007) IF: 1.1

Other articles:

1. Separation of Sesquiterpenes from Yarrow (Achillea millefolium L. s. l.) by LC-MS on Non-Porous Columns.
Gergely Montsko, Borbala Boros, Aniko Takatsy, Robert Ohmacht , Sabine Glasl, Liselotte Krenn, Laszlo Mark and Gottfried Reznicek
Chromatographia (67): 467-470 (2008) IF: 1.31

2. Analysis of pathological and non-pathological human skeletal remains by FT-IR spectroscopy.
Gergely Nagy, Tamas Lorand, Zoltan Patonai, **Gergely Montsko**, Istvan Bajnoczky, Antonia Marcsik, Laszlo Mark
Forensic Science International (175) 1: 55-60 (2008) IF: 1.86

3. Use of fullerene-, octadecyl-, and triaconthyl silica for solid phase extraction of tryptic peptides obtained from unmodified and in vitro glycated human serum albumin and fibrinogen.
Katalin Böddi, Anikó Takátsy, Szilvia Szabó, Lajos Markó, László Márk, István Wittmann, Róbert Ohmacht, **Gergely Montskó**, Rainer M. Vallant, Thomas Ringer, Rania Bakry, Christian W. Huck, Günther K. Bonn, Zoltán Szabó
Journal of Separation Science 32: 295 – 308 (2009) IF: 2.53

4. **9. Induction of mitochondrial destabilization and necrotic cell death by apolar mitochondria-directed SOD mimetics**
Aliz Szabo, Maria Balog, Laszlo Mark, **Gergely Montsko**, Zsuzsanna Turi, Ferenc Gallyas Jr.,Balazs Sumegi, Tamas Kalai, Kalman Hideg, Krisztina Kovacs
Mitochondrion (in press)
IF.: 4.15

Cumulative impact factor: 19.64

Oral presentations

1. Mass spectrometric analysis of non-derivatized steroids in biological matrices
Montsko G., Patonai Z., Kovács GL., Márk L.
6th International Medical Postgraduate Conference
Hradec Kralove, Czech Republic, 2009. November 19-21, p.: 89.

2. Archeome: Complex biomarker analysis of ancient human remains with MALDI TOF/TOF mass spectrometry
Mark L., **Montsko G.**, Nemeth V., Marcsik A
Second International Symposium on Biomolecular Archaeology
Stockholm, Sweden, 2006. September 7-9, p.: 34.

3. Determination of steroid hormones by matrix assisted laser desorption ionisation time-of-flight mass spectrometry with using fullerene as the matrix
Mark L., Makrai L., **Montsko G.,** Alexandra Vaczy
27th Informal meeting on mass spectrometry
Retz, Austria, 2009. May 3-7 p.: 25.

Posters

1. *Trans*-resveratrol and *trans*-piceid content of Hungarian wines
Montskó G., Jámbor É., Ohmacht R., Márk L.
15th International Symposium on Separation Sciences- Siófok, Hungary, 2009. September 2-4, p 198.

2. Bioaktív polifenolok analitikai viszgálata MALDI TOF tömegspektrometriával
Montskó G, Németh V, Pandur E, Nagy J, Márk L
36. Membrán-transzport Konferencia – Sümeg, 2006. May 23-26, p.: 60.

3. Szteránvázas hormonok kimutatása humán mintákból MALDI TOF tömegspektrometria alkalmazásával
Montskó G., Németh V., Pandur E., Nagy J., Márk L.
Magyar Biokémia Egyesület 2006. évi Vándorgyűlése Pécs, 2006. August 30- September 2, p.: 17.

Other oral presentations and posters

1. Paleoproteomics: determination of proteins and pathological biomarkers from bone remains, using MALDI TOF/TOF mass spectrometry
Montskó G, Németh V, Pandur E, Nagy J, Patonai Z, Márk L
XVI. Semmelweis Symposium & VI. Conference on Cell Analysis – Budapest, Hungary, 2006. May 4-6, p.: 112.

2. Biokompatibilis fullerén oldatok analitikai és toxicologiai vizsgálata
Montskó G., Vető S., Böddi K., Márk L
37. Membrán-transzport Konferencia – Sümeg, 2007. May 22-25 p.: 73.

3. Ajak és szájpadhasadék specifikus fehérjék kimutatása nyálmintákból MALDI TOF/TOF tömegspektrometria segítségével
Montskó G., Tihanyi R., Szabó T. Gy., Szabó Gy., Németh V., Márrk L
38. Membrán-transzport Konferencia – Sümeg, 2008. May 20-23, p:: 87.

4. Intracellular regulation of hepcidin expression
Pandur E., Nagy J., Szabó A., **Montskó G**., Radnai B., Sipos K
XVI. Semmelweis Symposium & VI. Conference on Cell Analysis – Budapest, Hungary, 2006. May 4-6, p:: 109.

5. The function of human RNase L Inhibitor in translation
Nagy J., Pandur E., Szabó A., **Montskó G** ., Bognár Z ., Sipos K
XVI. Semmelweis Symposium & VI. Conference on Cell Analysis – Budapest, Hungary, 2006. May 4-6, p.: 111.

6. MALDI TOF/TOF tömegspektrometria alkalmazása a jelátviteli folyamatokban jelentős kis molekulatömegű fehérjék azonosítására
Németh V, **Montskó G**, Vető S, Bognár E, Márk L
36. Membrán-transzport Konferencia – Sümeg, 2006. May 23-26, p.: 63.

7. A ferroportin egy hepcidin hormon által szabályozott vas transzporter
Nagy J., Pandur E.,. Szabó A., **Montskó G**., Bognár Z., Peti M. A., Sipos K
36. Membrán-transzport Konferencia – Sümeg, 2006. May 23-26, p.: 61.

8. A mitokondrium jelentősége a transzláció szabályozásában humán sejtekben
Pandur E., Nagy J., Szabó A., **Montskó G**., Sipos K
36. Membrán-transzport Konferencia – Sümeg, 2006. május 23-26, p.: 65.

9. A hepcidin, egy vasanyagcserét szabályozó hormon vizsgálata
Pandur E., Nagy J., **Montskó G.**, Peti M. A., Sipos K.
Magyar Biokémia Egyesület 2006. évi Vándorgyűlése Pécs, 2006. August 30- September 2, p.: 18.

10. Komplex biológiai minták GSH tartalmának meghatározása ioncsapdás tömegspektrometria segítségével
Németh V., **Montskó G**., Solti I., Vető S., Tucsek Zs., Hocsák E., Márk L.
Magyar Biokémia Egyesület 2006. évi Vándorgyűlése Pécs, 2006. August 30- September 2, p.: 20.

11. Az A-1 antitripszin szerepe a hepcidin érésében
Pandur E., Nagy J., Poór V. S., **Montskó G**., Sarnyai Á., Miseta a., Sipos K
39. Membrán-transzport Konferencia – Sümeg, 2009. May 19-22, p.: 105.

12. LC-MS analysis of green tea polyphenols
Éva Jambor, Balázs Marics, Simon Armbruszt, **Gergely Montskó**, Lászlo Márk, Róbert Ohmacht
15th International Symposium on Separation Sciences- Siófok, Hungary, 2009. September 2-4, p.: 190.