Mass spectrometric analysis of steroid hormones and other apolar molecules

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PhD thesis

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Pécs, 2016

Introduction

Steroids

Steroids belong to the lipid group of sterol lipids. All the natural steroids contain a fused four-ring sterane core structure. The basic sterol synthesized in animal cells is cholesterol. In the ovarium, different subclasses of sterols can be formed from it. Based on the number of carbon atoms in their structure there are C18 steroids (oestrogens), C19 steroids (androgens) and there is the C21 subclass, which includes progestogens, glucocorticoids and mineralocorticoids. Steroids along with the glycerophospholipids and sphingomyelins are important membrane components. Some of them are well-known endogenous hormones or have a role in the molecular signalling. Following the changes of steroid hormones in physiological or pathological states is a main focus of endocrinology. Steroids, however, can also be measured as environmental contaminants. Steroids can be categorised based on their functions as sex hormones, corticoids and anabolic steroids. I investigated only progestogens and oestrogenic compounds, which all belong to the group of sex hormones.

Progestins (also called progestogens, progestagens or gestagens) encompass progesterone (PRG) and structural relatives of it. There are also synthetic steroid drugs such as levonorgestrel (LNG) or drospirenone (DRO) which possess common progestogenic activity. The biological effects of individual progestins however can differ from each other. These progestins are widely used as synthetic oral contraceptives (SOCs), in hormone replacement therapy (HRT) and in the treatment of several gynecological disorders. According to literature data, more than 100 million women use SOCs every year. After oral, vaginal or other kind of administration progestins enter the blood stream and take effect. In the meanwhile, a certain amount of progestins will be metabolised. Metabolites and also unmetabolised drugs are eliminated from the human body mainly through the renal system. A remarkable amount is excreted unchanged or in the form of active sulfate or glucuronide metabolites. These metabolites also have pharmacological activity. Household derived contamination enters watercourses fortunately through waste water treatment plants (WWTP) where progestins are partially degraded. This decomposition however is not perfect, because these contaminants can be found in WWTP effluents. Progestins enter the aquatic environment not only through WWTP effluents but also through paper mill plant effluents and animal agricultural runoffs.

There is a continuously increasing amount of evidence about the influence of progestins on the hormone systems of aquatic species.

Oestrogens are principally known as female human sex hormones, but they can be found generally in vertebrate species and are physiologically present in males also. Oestrogens possess not only reproductive functions. They influence various cellular processes. The most potent natural oestrogen is β-oestradiol (E2). E2 is an endogenous steroid hormone present in both human and animal tissues and body liquids. E2 has a reputation as an effective medicine in menopausal/postmenopausal treatment (HRT), and in aging related diseases in woman. It is not only "the woman's hormone", thus tissues other than the female reproductive tract, such as the brain, are important targets of its action. In mammalian species both oestrogens, mostly E2 but others also, and androgens, such as testosterone (T), are principally synthetized in the gonads and in the adrenal glands. E2 is also produced from circulating steroid precursors, such as T, in several tissues, regardless of gender by aromatase enzymes (CYP19). The hypothalamus is most actively expressing aromatases in the brain, but moderate level is synthetized in the amygdala, hippocampus, midbrain and cortical regions in both rodents and humans Due to their intensive human therapeutic usage, E2 and EE2 are the most abundant steroid molecules with oestrogenic function, which can be found in the environment. In the European Union, pharmaceuticals authorization procedures include Commission directives, which contain a requirement to develop a strategic approach to the pollution of water by pharmaceutical substances. E2 and EE2 were newly added to the EU watch list of emerging pollutants in 2013 (Directive 2013/39/EU). They were also included in EU Commission Implementing Decision 2015/495 in which a watch list of substances has been established for Union-wide monitoring in the field of water policy pursuant to earlier Directive 2008/105/EC. There are several ways to analyse these pollutants. depending on the aim of the study and their opportunities analysts use GC-MS, LC-MS and immunoassays, but chromatography based methods with UV-VIS, fluorescence or electrochemical detection are also available. Recent developments in analytical chemistry enable the mass spectrometric detection of steroids even at the low ng/L level. There is an important difference in the MS ionisation of progestins and oestrogens. Progestins, probably through forming protonated ions, can be ionised easily in positive mode. In the case of E2 and EE2 deprotonation of a hydroxyl-group, thus ionisation in negative mode is observed commonly. Negative mode ionisation generally, and in the case of oestrogens too, enables only higher detection limits compared to positive mode methods. Sensitivity can be though increased in mass spectrometry by chemical

transformation of the analyte. Derivatisation of E2 and EE2 prior to mass spectrometric analysis makes positive mode detection possible, which improves both sensitivity and specificity of their analysis. Full MS and targeted MS/MS methods without derivatisation, which are generally used in environmental monitoring applications, detect 40-100 or even more target compounds. These screening methods however often do not include or cannot detect E2 and EE2 due to poor detection limits. A novel comparison of derivatisation methods for the determination of E2 using standard solutions was recently published. We applied dansyl chloride (DSCl) derivatisation in our method because it was found to be very sensitive and DSCl is approximately 10 fold cheaper than 1-methylimidazole-2-sulfonyl chloride, which produced the lowest limit of detection in the comparison.

Carotenoids

Carotenoids are strongly apolar prenol lipids. They possess a C_{40} carbon skeleton built up from 8 isoprene unit forming a linear molecule. They are lipid antioxidants in human, plant and in animal organisms and some of them are fat soluble provitamins. β-carotene and its derivatives contain unmodified β-ionone end groups, and they serve as precursors for vitamin A and are therefore essential dietary components for humans and for mammals in general. Plants are a natural source of colourful carotenoids. Pigmentation is caused by the chromophore part, the conjugated double bond system of the molecule. The higher the number of the conjugated double bonds is, the higher the absorbance and the longer the wavelengths of the absorbed light will be, causing a more reddish hue of carotenoids. Carotenoids can be divided into two major groups: carotenes and xanthophylls. Carotenes consist of only carbon and hydrogen atoms (e.g., α-, β - and γ - carotenes and lycopene), while xanthophylls are oxygenated derivatives of carotenes containing hydroxyl-, keto-, epoxy- and methoxy- groups. The number of known natural carotenoids is over 700 and carotenoid content of different plants have a high variability. HPLC-UV-VIS or HPLC-ECD methods applying C_{18} or C_{30} stationary phases are generally used for the determination of these compounds. In mass spectrometric studies of such non-polar analytes as carotenoids APCI ion sources are dominant, but the softer ESI ionization is also capable for their analysis.

Aims

Steroid measurements

1. Development of a simple, fast, sensitive and relatively inexpensive method for the simultaneous detection and quantification of DRO, LNG and PRG from freshwater samples.

2. Development of rapid and sensitive methods for the determination of E2 and EE2 from water samples as environmental pollutants and from blood and brain tissue (E2 only) as endogenous hormone.

3. Survey of the endogenous brain E2 levels of mice including regional differences.

4. Providing reliable data about the actual level of contamination regarding these steroids in Hungarian and other freshwaters

Structural elucidation of carotenoids

5. Identification of β-cryptoxanthin-epoxides extracted from Red Mamey (*Pouteria sapota*)

Sample preparation

Environmental studies

Sampling of rivers, watercourses and Lake Balaton

We collected samples in Slovenia and in Hungary, from Lake Balaton, from six Central-European rivers (Danube, Drava, Mur, Sava, Tisza and Zala), from smaller watercourses and from a city canal in the urbanized area of the city of Pécs. Samples have been collected from the upper 30 cm surface layer of freshwater within 2 m distance from the bank unless otherwise described with coordinates. Two main environmental studies are described in the thesis. The first study was achieved in July 2014. In this earlier study progestins have been investigated in freshwater at 53 sampling points in the catchment area of Lake Balaton, Hungary. In the second study, which was achieved in July 2015 the occurrence of oestrogens (E2 and EE2) has been investigated in all the large rivers of Central-Europe (21 sampling points The volumes of the analysed samples have been: 770 ml in the first study (progestins), 1000 ml freshwater in the second study (oestrogens) and only 500 ml in the case of Pécsi víz city canal water (PV) in the second study.

Drug residues from water samples were concentrated on Strata C18-E (1g/20 mL) SPE cartridges. After the solid phase extraction in the case of progestin measurements extracts were reconstituted in methanol. In the case of oestrogen measurements derivatising agent (dissolved in acetone) was added to them and they were derivatised.

Mouse brain and blood E2 study

Animals

Adult female mice (C57BL6) were bred and housed at the University of Pécs. The animals were maintained under conditions of 12-h light, 12-h dark cycle (lights on at 0800 h) with food and water available *ad libitum*. All protocols and procedures were approved and performed in accordance with approved protocols (University of Pécs; BA02/2000-15024/2011). Control and ovariectomized (OVX) animals have been used. The latter were bilaterally ovariectomized under isoflurane anesthesia and used for experiments 2 weeks later.

Animals were sacrificed with decapitation, blood was collected in heparinized Epppendorf tubes and brains were removed and dissected in a sagittal axis, which resulted in 500 µ thick coronal sections. Numbering of the sections starts with No. 1 at the nasal end of the brain, numbers are increasing to the caudal direction.

Preparation of dansyl-derivates

E2 and EE2 standards and dried sample extracts were treated similarly. 50µL 0.2M sodium bicarbonate (in LC-MS grade water) and 50 µL 1 mg/mL dansyl chloride (in acetone) were added to each of them. These mixtures were incubated in a thermomixer (65°C, 300rpm) for 10 minutes. Then they were cooled down on ice (2 minutes) and transferred to the autosampler of the HPLC, which was kept at 4°C.

HPLC-MS/MS methods

Analysis of DRO, LNG and PRG as environmental contaminants

5 µL was injected 3 times from each concentrated sample into the HPLC-MS system. Liquid chromatographic separation was carried out on a Kinetex 2.6 µ C18 100Å HPLC column (100*2.1 mm) maintained at 40°C. The mobile phase consisted of solvent A (0.01 v/v% formic acid in water) and solvent B (0.01% v/v% formic acid in acetonitrile). The flow rate was 300 µL/min. Initial gradient conditions were set to 35% B and held for 3 min then B was increased linearly reaching 55% at 12 min, then initial conditions were reached in 0.2 min and the column was equilibrated for 7.8 min.

The mass spectrometer was equipped with a heated electrospray ion source which was operated in positive ion mode. Spray voltage was set to 4.0 kV. Capillary temperature was 300 °C. The probe heater temperature was 50 °C. S-lens RF level was set to 70. Different HCD (Higher-Energy Collisional Induced Dissociation) cell energies were applied for the fragmentation: 55% by DRO and 45% by PRG and LNG. Data analysis was carried out with the software Thermo Xcalibur (version 2.2 SP1.48).

The observed ions were accepted if their m/z value were within the following limits: DRO - MS1: 367.21-367.24, MS2: 97.06-97.08, retention time: 5.2±0.25 min; LNG - MS1: 313.20-313.23, MS2: 109.05-109.07 and 245.18-245.20, retention time: 5.7±0.25 min; PRG - MS1: 315.21-315.24, MS2: 97.06-97.08 and 109.05-109.07, retention time: 9.8±0.25 min. ICIS peak detection was achieved using Xcalibur (area noise factor: 5.0, peak noise factor: 10.0 and minimum peak heights 3.0 (S/N

Analysis of E2 and EE2 as environmental contaminants

10 µL was injected three times of each derivatised sample. Liquid chromatographic separation was performed on a Kinetex 2.6u XB-C18 100Å HPLC column (150x2.1mm) maintained at 30°C. The mobile phase consisted of solvent A (0.01% v/v formic acid in water) and solvent B (0.01% v/v formic acid in acetonitrile). Flow rate was set to 400 µL/min. The initial composition contained 10% B and it was kept constant for 1 minute. Percentage of eluent B was raised to 25 in 2 minutes. Then it was raised further to 70% in 2 minutes, then to 75% in 10 minutes. After that the column was washed and equilibrated for 15 minutes. The chromatographic peak of E2 was observed at 13.09-13.17 min, EE2 was eluted at 13.66-13.73 min. Mass detection was carried out in positive mode. Spray voltage in the heated electrospray ion source was set to 4.0 kV Capillary temperature was set to 380°C, while the probe heater temperature was 300°C. RF of the S-lenses was set to 60. Sheath and auxiliary gas flow rates were set to 60 and 20 arbitrary units respectively. No sweep gas was applied. The energy in the higher-energy collisional induced dissociation (HCD) cell was set to 50% by E2 and 49% by EE2. Automatic gain control was set to 1e6 by MS1 and 2e5 by MS/MS scans. tSIM scan ranges were 506.0-506.5 and 530.0-530.5 m/z. By targeted-MS/MS scans we applied a 0.4 m/z isolation window and 506.24-171.10 (E2) and 530.24-171.10 (EE2) transitions (m/z) have been used.

Analysis of endogenous E2 in blood and in brain tissue

25 µL was injected three times of each derivatised sample. Liquid chromatographic separation was performed on a Kinetex EVO 5u C18 100Å HPLC column (150*2.1mm) maintained at 30°C. The mobile phase consisted from the same A and B solvents which were described by the environmental oestrogen method. Flow rate was set to 300 µL/min. The initial composition contained 75% B and it was kept constant for 1 minute. Percentage of eluent B was raised to 80 in 2 minutes. Then it was raised further to 99% in 2 minutes, and then composition was kept constant for 5 minutes. After that the column was washed and equilibrated for 15 minutes. Settings of the mass spectrometer were the same as applied by environmental oestrogen measurements. Brain tissue samples have been spiked with either 50 pg EE2 prior to homogenization, EE2 was used as internal standard in data processing.

Results and discussion

Steroid measurements

Environmental analysis of steroids

DRO, LNG and PRG

In progestin measurements less than 1ng/L LOQ (limit of quantitation) has been reached from less than 1 L freshwater. The analysis was achieved in a relatively environmentally friendly way, because it did not require chemical derivatisation.

Progestin concentrations were variable among the 53 collecting places around Lake Balaton. 23 of the freshwater samples did not contain measurable amount of contamination (<LOQ, not shown), however at 21 collecting places we observed steroids. Out of this 21 freshwater sample in 20 PRG was detected. LNG was found at 6 (No 1, 5, 11, 12, 20, 21) and DRO was found at 5 places (No 1, 5, 11, 19, 21). We observed 4 places (No 1, 5, 11, 21) where all the three steroids were present. Measured progestin concentrations were found [in ng/L] 0.26-4.30 (DRO), 0.85-3.40 (LNG) and 0.23-13.67 (PRG).

E2 and EE2

E2 and EE2 without derivatisation can only be detected with poor LOQs. Achieving negative mode measurements, I found the following LOQs: 7.5 µg/L (E2) and 20 ng/L(EE2). Applying derivatisation we reached 0.05 ng/L (E2), and 0.001 ng/L (EE2) LOQs. These methods are also fast and simple, however, they are less environmentally friendly than the progestin method. Both E2 and EE2 were found in river samples. EE2 was less abundant than E2. n.d.-5.8 ng/L E2 and n.d.-0.7 ng/L EE2 concentrations were measured. A relative high amount of EE2 was found in River Zala (0.7ng/L) and in Hévíz-Páhoki canal (0.5ng/L), which are both in the catchment area of Lake Balaton. The presence of E2 could be confirmed with MS/MS transitions in 12 out of 23 samples. The presence of EE2, due to poor (0.2ng/L) LOQ value, could only be confirmed with MS/MS transitions at two sampling sites (Hévíz-Páhoki canal; HP and River Zala; ZA). In the case of five samples we repeated the measurements to determine, how much oestrogen can be extracted from filtered samples and from the filter papers separately. From the extracted amounts I calculated an estimation of proportionality of E2 and EE2 between suspended and dissolved phase. Smaller amounts (17-33% E2 and 0-5% EE2) could be extracted from the suspended phase and larger amounts from the liquid phase, which according to literature data is unexpected, most steroids show the opposite proportionality.

Due to the intensive use and high demand of estrogens, the occurrence of E2 and EE2 in natural waters is a major concern. According to data published between 2003 and 2013, scientists found more than 1 ng/L E2 at least at one of their sampling sites in 13 out of 18 freshwater investigations worldwide. EE2 could be detected over 1 ng/L with a 50% chance. EU regulation has put E2 and EE2 on the watch list of emerging pollutants in 2013 and in this year they established maximum acceptable detection limits for them. These limits are 0.035 ng/L for EE2 and 0.4 ng/L for E2. These contaminants in an average equipped laboratory using multiresidue analysis without derivatisation can only be determined with poor detection limits. Our methodology with dansyl derivatisation is cheap, quick and simple enough to be considered for use in monitoring studies in the future. The use of 1-methylimidazole-2-sulfonyl adducts may be another possibility, but it must be tested. Compared to the international dataset , E2 and EE2 contamination in rivers of the Carpathian basin and Lake Balaton is generally moderate. Even the concentrations measured at capital cities (3.0 ng/L E2 and 0.1 ng/L EE2 at Budapest and 5.2 ng/L E2 at Ljubljana) seem to be tolerable. Though it

must be taken into account that very limited data is available, that concentrations can raise dramatically if passage flow decreases, and that most toxicological experiments are achieved during a few weeks or months, thus long-term effects are very hard to discover. This low level presence of E2 and EE2 in natural waters is just a small contributions to the overall oestrogenity of waters (Wise, et al. 2011), which at its actual level can cause local problems in the wildlife but probably means minimal risk to public health.

Mouse brain and blood E2 study

Original fast and simple LC-MS methods have been developed for the measurement of E2 from blood and brain samples. E2 content of dissected brain cortex regions of adult female mice and plasma has been measured. Plasma concentration have been compared with oestrus phases determined via vaginal cytology. Measured plasma E2 concentration were well-fitted with oestrus phases. E2 plasma concentrations of intact and OVX mice have been compared. The bilateral removal of the ovaries ablates the gonadal E2 production that way brain concentrations can be investigated minimising the influence of circulating E2. (Adrenal glands still fulfil their functions.) Plasma E2 concentrations of OVX animals after two weeks of the surgery were found detectable (1-3 pg/mL), but 10-75 fold lower than the normal level.

E2 could be quantified from less than 5 mg brain tissue sample. As an average mouse brain is approximately 350-450 mg the technique allows a limited mapping the E2 content of mice brains. The limit of the quantification (LOQ) of local concentration of the hormone in this assessment is 0.05 pg E2 on column or 0.2 pg E2 in sample. It is equivalent with 0.04 pg/mg if we have 5 mg brain tissue, but of course we have a lower pg/mg limit if we analyse a larger tissue section and a higher limit by the analysis of a smaller tissue section. Hypothalamus, hippocampus and cortex sections have been analysed (n=9). I have measured the following average E2 concentrations: hypothalamus: 0.20-50 pg/mg, hippocampus: 1.0-155 pg/mg, cortex: 0.25-6.2 pg/mg. In the case of cortex sections left and right sides have been investigated separately (Figure 1). Estradiol concentration in the left side and in the right side were compared. Proportionality (left side E2 concentration/right side E2 concentration ratio) was found smaller than 0.5 in 1 brain, between 0.5 and 1.5 in 4 brains and over 1.5 in 4 brains.

Coronal brain sections were cut and local E2 concentrations were investigated in separate samples (mice numbered from 10^{th} to 14^{th}) on a sagittal (nasal to caudal) axis of mouse brain

cortices also (n=5). These samples showed similar average E2 concentrations to concentrations measured in mice 1st-9th, but large local differences could also be observed.

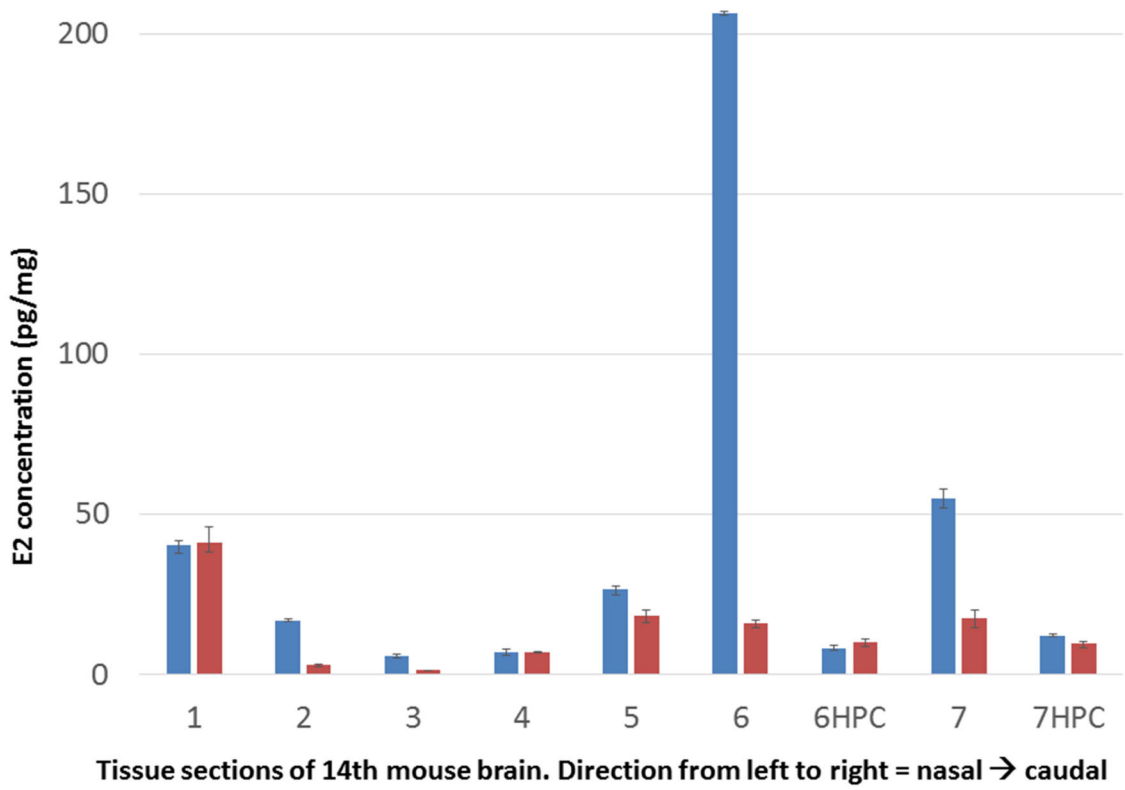

Figure 1. Local E2 concentrations in the sections of a mouse brain (Mouse No. 14th). Numbers 1-7 mean cortices of brain section 1-7. Section No. 1 is the nasal end of the cortex, section No. 7 is the caudal end of the cortex. 6HPC and 7 HPC are hippocampus sections cut from brain sections 6 and 7.Blue: left side, Red: right side

The total number of investigated mice (n=14 normal brain, n=5 normal plasma, n=1 OVX plasma) does not enable us to claim well established conclusions about the observed phenomenon, that E2 concentration were not the same in the right and left sides of hippocampi and cortices, but it raises the question whether differences are significant. If E2 concentrations differ on the left and right sides of brain structures many results of previous experiments become more problematic to comprehend. Studies where the two hemisphere of brain tissue are used for different type of analysis, for example one hemisphere for immunohistochemistry and the other hemisphere for molecular biological analysis must be planned more carefully. We continue this study in order to answer this and several further questions.

Structural elucidation of carotenoids

The carotenoid research group of our institute provided me two chromatographic fractions. Both were identified as β-cryptoxanthin epoxides based on their UV-VIS spectra. The component collected from the chromatographic separation at the retention time 14.5 min had the mass 568.43 (m/z). That m/z matches with the calculated theoretical mass of a β-cryptoxanthin epoxid. Fragmentation of this m/z even with relatively low fragmentation energies produced a large number of ions. The fragmentation profile was very similar to earlier reported fragmentation of β-cryptoxanthin. Characteristic ions were 553.40 ((M-15) or β-cryptoxanthin), 488.36 (M-C_6H_8), 476.36 (M-C_7H_8), 461.34(β-cryptoxanthin-C_7H_8), 415.30 (M-153, loss of the hydroxylated ring with cleavage at the 7,8 carbon-carbon bond from the protonated molecule) and several fragment ions corresponding to cleavages of the polyene chain.

The other component, collected from the chromatographic separation at the retention time of 17.5 min had the same mass 568.43 (m/z). The fragmentation of this compound seemed to be almost identical to the fragmentation of the other compound with the retention time 14.5 min, it contains the same high abundant ions, however not the same ions are the most intensive ones. To tell the position of the epoxidation (Figure 2) the theoretical masses of two ions (169.12340 and 171.13769) (Figure 3) have been calculated, which both contained the six membered ring, four methyl groups, the epoxide and the hydroxyl groups as they all together could only occur in the tandem spectra if the position of the epoxidation is 5,6. All the tandem spectra were analysed using mass isolation ranges 169.11-169.13 and 171.13-171.15. These two ions were only observed in the spectra of the compound with the retention time 17.5 min Their intensities applying 25% HCD cell energy were 3 and 6 % of the intensity of the precursor ion (568.43) or 0.01 and 0.02 % of the total ion chromatogram.

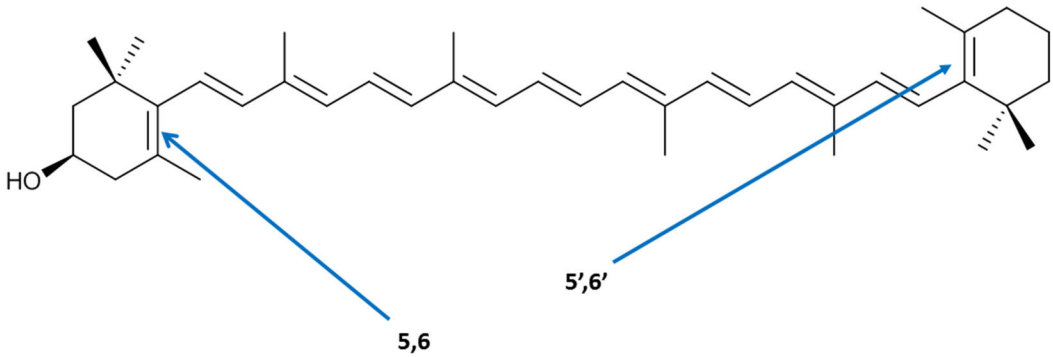

Figure 2. Possible sites of epoxidation on β-cryptoxanthin. Epoxid formation is possible either on the 5,6 carbon-carbon bond or on the 5',6' carbon-carbon bond.

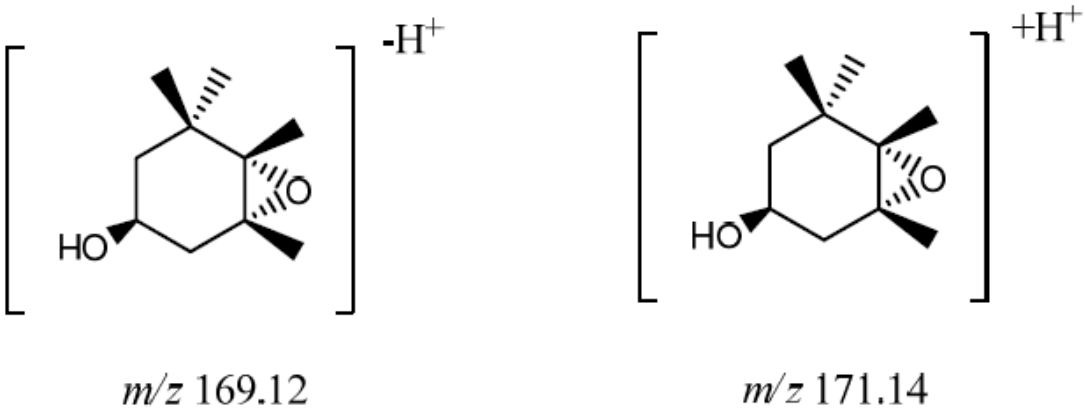

Figure 3. The two ions, 169.12 (left) and 171.14 (right) which were used for the differentiation of 5,6 and 5',6' epoxides.

Summary

In the present doctoral thesis new mass spectrometric techniques have been developed and the analysis of steroid lipids and carotenoid lipids was achieved.

Steroid methods yielded quantitative determination of important progestogens and oestrogens and they suited for the analysis of both environmental samples and medical research samples. The occurrence of drospirenone (DRO), levonorgestrel (LNG) and progesterone (PRG) as environmental contaminants have been surveyed in freshwater samples from the catchment area of Lake Balaton. 17β-oestradiol (E2) and 17α-ethinyl oestradiol (EE2) have been determined in Lake Balaton and in all the large European rivers. DRO, LNG and PRG were analysed at 53 sampling points. They could be analysed in their original form. E2 and EE2 were analysed in waters collected at 23 sampling points, they had to be derivatised and were detected as dansyl-derivates. Derivatisation was necessary to reach sufficient limits of detection. The concentrations of contaminants showed variability, but they were found generally moderate, in the low ng/L range.

Endogenous E2 levels in mouse brain tissue samples (n=14) have been surveyed to support further research. Average concentrations in full cortices were 0.25-6.2 pg/mg, but great local differences have been observed. The analysis of whole cortices, cortex sections and hippocampi all raised the question whether lateralisation of the brain in the context of different hemispherical E2 levels exist.

The qualitative analysis of carotenoid extracts proved, that ESI-MS is suitable for the structural elucidation of such apolar molecules. The position of epoxidation of β-cryptoxanthin was successfully identified.

Results have demonstrated that LC-MS is an excellent tool for determining low ng/L environmental contaminants in freshwater, low pg/ml E2 in plasma nd low pg/mg E2 in brain tissue. Preconcentration due to low analyte concentrations is indispensable in both environmental and medical research methods. Solid phase extraction has shown great potential in preconcentration of water samples while effective liquid/liquid extractions have been developed for tissue and plasma preconcentration.

Novel findings

1. Original, sensitive and fast methods based on SPE and LC-MS have been developed for the determination of the following environmental contaminants: DRO, LNG, PRG, E2 and EE2.

2. A significant steroid hormone contamination including quantitative data was measured in the catchment area of Lake Balaton and in several Central European rivers.

3. A sensitive method has been developed for the determination of E2 from brain tissue, physiological concentrations in the mouse brain have been surveyed.

4. Using MS/MS experiments the identity of 5,6 and 5',6' β-cryptoxanthin-epoxides was successfully confirmed and I could tell them apart.

Acknowledgements

I would like to thank the Program Director, Prof. Balázs Sümegi, for letting me enter the PhD program. I would like to thank my supervisor Dr. László Márk from the University for his support. He built up a state-of-the-art mass spectrometric laboratory, encouraged me to enter the PhD program and he enabled me to develop independent thinking. I would like to express my special appreciation and thanks to my supervisor Dr. Zsolt Pirger from the Balaton Limnological Institute. He has been a tremendous mentor for me. He encouraged several of my ideas, and he was always inspiring. He won the grant KTIA_NAP_13-2-2014-0006, which supported the environmental studies. I would like to thank Prof. Dr. Zoltán Juvancz for his valuable insights in the topic of oestrogens. I am grateful for Dr. János Pálhalmi, Prof. István Ábrahám and for the whole Molecular Neuroendocrinology Research Group of the Szentágothai Research Centre for the intensive cooperation. The mouse brain investigations, where I measure E2 is an ongoing study, and it is a part of a larger project achieved by the Molecular Neuroendocrinology Research Group and supported by the State of Hungary under the grant KTIA_NAP_13-2014-0001. I would like to thank Prof. József Deli, who invited me to participate in his carotenoid research project, which project also contributed to my thesis. I am grateful for the opportunity to use the laboratory of Dr Attila Agócs, where I prepared samples in the environmental studies. I appreciate that Gergely Gulyás, Dr. Gábor Maász, Dr. Anikó Takátsy and János Schmidt helped me in the editorial work. I am grateful for Erika Turcsi for the many things in which she has helped me. I would like to thank to my family and all of my co-authors for their support in my experimental work. I appreciate the help of all my colleagues from the Institute of Biochemistry and Medical Chemistry.

List of Publications

Thesis related publications

Communications in scientific Journals

- ***Avar P***, Zrínyi Z, Maász G, Takátsy A, Lovas S, G.-Tóth L, Pirger Z (2016) β-estradiol and ethinyl-estradiol contamination in the rivers of the Carpathian Basin Environ Sci Pollut R, DOI: 10.1007/s11356-016-6276-2 IF: 2.828
- ***Avar P***, Maász G, Takács P, Lovas S, Zrínyi Z, Svigruha R, Takátsy A, G.-Tóth L, Pirger Z (2016)
  HPLC-MS/MS analysis of steroid hormones in environmental water samples Drug Testing and Analysis, 8: 123-127. IF: 2.506
- Turcsi E, Murillo E, Kurtán T, Szappanos Á, Illyés TZ, Gulyás-Fekete G, Agócs A, ***Avar P***, Deli J (2015)
  Isolation of Beta-Cryptoxanthin-epoxides, Precursors of Cryptocapsin and 3'-Deoxycapsanthin, from Red Mamey (*Pouteria sapota*) J Agr Food Chem, 63: 6059-6065. IF: 2.912

Lectures

- ***Avar P***, Maász G, Takács P, Lovas S, Zrínyi Z, Svigruha R, Takátsy A, G.-Tóth L, Pirger Zs (2015)
  Szteroid szennyezõk tömegspektrometriás vizsgálata a Balaton vízgyûjtõ területén XII. Környezetvédelmi analitikai és technológiai konferencia, Balatonszárszó, Hungary
- Pirger Z, Takács P, Bévárdi N, Svigruha R, Maász G, ***Avar P*** (2014)
  Humán eredetű szteroid terhelés és annak lehetséges élettani hatásai a Balaton és a Zala vízgyűjtőjén IV. Ökotoxikológiai Konferencia, Budapest, Hungary
- Pápai Z, Maász G, Schmidt J, ***Avar P***, Márk L (2014)
  Lipidek időbeli és térbeli változásainak feltérképezése a korai embriogenezis során III. Interdisciplinary Doctoral Conference, Pécs, Hungary

Posters

- Pálhalmi J, ***Avar P***, Ábrahám M. I (2016)
  Single molecule imaging of AMPA receptor in live neural network of adult mouse

brain, International Brain Research Organization (IBRO) Workshop, Budapest, Hungary

- ***Avar P***, Maász G, Zrínyi Z, Takátsy A, Pirger Z (2015)
  Quantitative measurement of 17 ß-estradiol and Ethinyl estradiol in some Central-European rivers, European Meeting on Environmental Chemistry (EMEC 16), Turin, Italy
- ***Avar P*,** Maász G, Takács P, Svigruha R, G.-Tóth L, Pirger Z (2015)
  HPLC-MS/MS analysis of steroid hormones in environmental water samples,

  International Symposium on Separation Sciences (ISSS), Ljubljana, Slovenia

Other publications

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- Kuzma M, Fodor K, Maász G, ***Avar P***, Mózsik G, Past T, Fischer E, Perjési P (2015) A validated HPLC-FLD method for analysis of intestinal absorption and metabolism of capsaicin and dihydrocapsaicin in the rat. J Pharmaceut Biomed, 103: 59-66. IF: 2.867
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- ***Avar P***, Nikfardjam MPS, Kunsági-Máté S, Montskó G, Szabó Z, Böddi K, Ohmacht R, Márk L (2007)

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  16. Labortechnika Kiállítás, Tömegspektrometriai Szakmai nap, Budapest, Hungary
- Zelenyánszki D, Schmidt J, Pápai Z, Maász G, ***Avar P***, Márk L (2014)
  Tumormarkerek azonosítása MALDI imaging képalkotó technika segítségével
  III. Interdisciplinary Doctoral Conference, Pécs, Hungary
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  HPLC-MS analysis of capsaicin, dihydrocapsaicin and their metabolites
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Poster

- ***Avar P*** (2015)
  Cell energetics with HPLC-MS
  33rd Informal Meeting on Mass Spectrometr**y,** Szczyrk, Poland