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Editorial

Steroid LC–MS has come of age

It now seems to be an opportune time to devote a special issue of the *Journal of Steroid Biochemistry and Molecular Biology* (JSBMB) to the application of liquid chromatography–mass spectrometry (LC–MS) to steroid research. In this analytical technique, detection is enabled by mass spectrometry, which presents a truly remarkable scientific tool. Its principle consists in generating multiple ions from a sample, separating these ions in an electromagnetic field according to their mass-to-charge ratio (m/z), and finally, recording of the relative abundance of each ion. Meanwhile, mass spectrometry has developed into the perhaps most versatile and exact of all analytical techniques permitting generation of qualitative as well as quantitative data on the atomic and molecular structure of inorganic and organic materials [1].

May the editors remind the inclined reader that mass spectrometry is a technique with fine tradition? Its fundamentals had been discovered and applied by the ground breaking work of J. J. Thomson – Nobel Prize in Physics 1906 – and his research assistant F.W. Aston – Nobel Prize in Chemistry 1922 – over 100 years ago [2,3]. Which other technique has since then undergone such enormous progress and refinement, and has been awarded so many more Nobel prizes? In physics, W. Paul and H.G. Dehmelt received them in 1989 for the development of the ion trap. Subsequently another major breakthrough consisted in the development of "soft", i.e. non-disintegrating ionization techniques: in 2002, J.B. Fenn and K. Tanaka were awarded Nobel prizes in chemistry for electrospray and soft laser desorption ionization methods, respectively. These techniques overcome the propensity of complex molecules to fragment when ionized and allow for the production of protonated $[M+H]^+$ or deprotonated $[M-H]^-$ molecules (formerly called pseudomolecular ions; [4]) without extensive fragmentation. These achievements opened up new vistas for the analysis of biomolecules.

The combination of mass spectrometry with separation methods such as gas chromatography (GC) or LC (the acronym HPLC stems from the expression "high pressure liquid chromatography", a term coined by C. Horvath in 1970) leads to so called "hyphenated techniques". These provided a further unique advantage: the simultaneous and unbiased, i.e. non-discriminatory determination of multiple analytes in a single run. This feature of multicomponent analysis started a renaissance in metabolism research. It paved the way for the field of metabolomics, which is the systematic study of small molecule metabolites characterizing a biological sample [5].

Accordingly, mass spectrometry has also proven an invaluable tool regarding research into steroids. Steroids represent a class of relatively small molecules essential for practically all forms of life. They are indispensable for the formation of cell structures, and often act as signaling molecules, constituting an elaborate and highly important information transfer system. While GC–MS had its start as a highly successful research tool mostly for unconjugated steroids already in the sixties and seventies of the last century [6,7], it took about further 20 years for LC–MS to follow.

At that time, it had been the privilege of one of the editors of this special edition (Stefan A. Wudy) to witness the earliest steps in the life of this "newborn" technique as a postdoctoral fellow in the steroid research laboratory of Professor Cedric H.L. Shackleton at Children's Hospital Oakland Research Institute (California, USA). Shackleton, a pioneer in mass spectrometric steroid analysis, had just made use of the short interplay of fast atom bombardment (FAB) in the analysis of conjugated steroids without derivatization [8], when his group could subsequently show the applicability of Thermospray LC–MS [9]. The latter technique proved particularly suitable for the analysis of intact sulfated steroids [10]. These developments heralded a revolutionary development in the field of mass spectrometric steroid analysis. In the meantime, the availability of stable isotope labeled internal standards [11] as well as further technical improvements have led to an increased use of LC–MS based methods for analyzing many classes of steroids. Currently, electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are the prevailing soft ionization techniques.

Introduction of tandem mass spectrometry (MS/MS), especially the triple quadrupole systems [12], represented a further breakthrough in LC–MS, since this technique compensates for the rather poor chromatographic capacity of LC (in comparison with GC). For instance in clinical steroid analysis, Wudy et al. could show that diagnostically important steroids, e.g. 17-hydroxyprogesterone, were not only amenable to GC–MS [13] but also to LC–MS/MS [14]. Since then, the technique has revolutionized clinical steroid analysis. Sooner or later will the problem of lacking specificity of immunoassays, particularly of cheap and direct assays, be overcome by mass spectrometry based methods. Additionally, LC–MS/MS allows for high throughput analysis since only simple sample preparation is required and instrumental run times are short. Furthermore, regarding complex, e.g. conjugated steroids, the LC–MS/MS approach currently presents the method of choice [15]. During the last years, LC–MS/MS has really come of age with dramatic improvements in sensitivity, specificity and automation.

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Little wonder that the broad applicability of LC–MS is also reflected by the diverse composition of the team of this special edition's guest editors. Both, Stefan Wudy, a pediatric endocrinologist, and Man-Ho Choi, a bioanalytical chemist, have been either directly or indirectly been influenced by Shackleton and are both passionate steroid mass spectrometrists. They were enthusiastic about the invitation and privilege to edit this special issue at this time. It is the intention of this special edition to demonstrate the utility of current LC–MS technology in steroid research in its broadest sense. The guest editors were happy that, after the call for proposals, a great response from many world leading authorities arose to contribute articles for this special issue.

The reader of this special issue will notice that LC–MS can be applied to practically all classes of steroids. Oxysterols, which are oxidized products of cholesterol, are increasingly recognized as important players in a variety of diseases such as atherosclerosis, cancer or several neurological disorders, while glucocorticosteroids are associated with adrenal functions and pharmacological uses. Due to their low concentrations and oxidizability, they are not easy to analyze. The comprehensive overviews by Griffiths et al., as well as by Hawley and Keevil, provide highly valuable contribution how to reliably determine these compounds by LC–MS.

By no means is steroid metabolism limited to the compounds that have up to now been enclosed in the "canonical" pathways to be found in our textbooks! More and more new steroids, either unconjugated or conjugated are being discovered and all readers interested in learning how to identify them are being referred to the highly informative review of Marcos and Pozo, who introduce LC–MS as an indispensable tool for this task.

Due to improving the volatility and the thermal stability with better selectivity and sensitivity in MS detection, the chemical derivatization was mainly used in GC–MS analysis, but it has been also considered in LC–MS-based steroid analysis. Higashi and Ogawa introduce the chemical derivatization techniques for steroid analysis with ESI, which have better sensitivity over APCI. The group of Blair focused more on the advantages of chemical derivatization onto the ultra-high sensitive estrogen analysis with LC-ESI–MS/MS and reviews both assay consideration and suggested practices.

Nowadays doping analysis is unimaginable without LC–MS! The group of Thevis demonstrates the elucidation of the metabolism of an anabolic steroid using current MS techniques.

In contrast to the more classical biological matrices such as biological fluids (e.g. blood, urine) or tissues, hair is a relatively recent target in steroid analysis. As hair grows at a pace of approximately 1 cm/month, it allows for obtaining condensed information on the steroid hormonal milieu over a relatively long time. First successful attempts of mass spectrometric steroid determination in hair have been made at the end of the nineties of last century using GC–MS [16,17]. The facettes of LC–MS based determination of steroids in hair are summarized in the highly interesting review by the group of Stalder.

In 1997, the World Health Organisation has proclaimed childhood obesity a global epidemic. Since then enormous efforts have been made in the field of obesity research to elucidate its causes and consequences. In the search for new agents of metabolic syndrome, Choi's group developed a method for simultaneous profiling of steroid conjugates with sulfuric and glucuronic acids in serum and informs about its application to a cohort of obese children.

One of the major steroid classes are adrenal steroids and Soldin and collaborators report an improved LC–MS micro-method in the atmospheric pressure photoionization (APPI) for steroid profiles applicable into adrenal insufficiency and congenital adrenal hyperplasia.

Bile acids are actually steroid acids and typically found in mammals and vertebrates. While their synthesis in the liver, excretion in bile, further modification by bacteria in the gut, and reabsorbtion within the enterohepatic circle, has for long been known, their detection in ovarian fluid is a very recent finding [18]. To further investigate their role in an animal model (bovine ovaries), an efficient LC–MS method, has been developed by Wudy's group. In addition, Kushnir et al. report here altered steroid profiles in stimulated human ovarian follicular fluids with outcomes of IVF treatment.

Both editors of this special edition are indepted to two of Stefan A. Wudy's coworkers, Alberto Sánchez-Guijo (M.Sc.), and Michaela F. Hartmann (Ph.D.), for their fabulous bibliographic and administrative help. Furthermore, the editors thank the chief editor of this journal, Professor Jerzy Adamski (Munich, Germany) for providing the kind invitation and opportunity to issue this special edition. The editors are grateful to all contributing authors for their spontaneous will to participate! Thank you so much, your contributions are outstanding!

Finally, Stefan A. Wudy and Man Ho Choi devote this special issue to all those who are new in the field. Might they also get fascinated by steroids, these small universal molecules, and the unraveling of their secrets by LC–MS. And of course, this edition is also devoted to the more experienced ones working already in this exciting field! All editorial members of JSBMB hope that this edition might allow all those interested in the field of steroid LC–MS to get a quick update on what is currently going on "in the scene".

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