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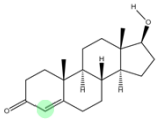
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OBJECTIVES

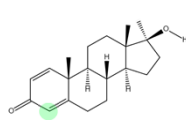
The test method is intended to be used for the qualitative and quantitative determination of various anabolic steroids, prohormones and anti-estrogens as pure active ingredients as well as quality parameters in tablets or injection oils. This method is designed to replace the previously used HPLC method which is quite time consuming and needs expensive reference substances with limited storage life. The NMR method is expected to be greener, faster and cheaper.

STUDIED MOLECULES

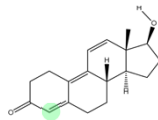
Steroids



Testosterone

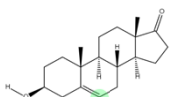


Methandienone

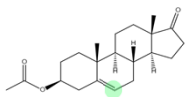


Trenbolone

Prohormones

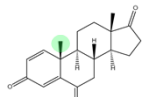


DHEA

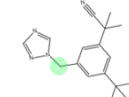


DHEA Acetate

● proton(s) used for quantification



Exemestane



Anastrozole

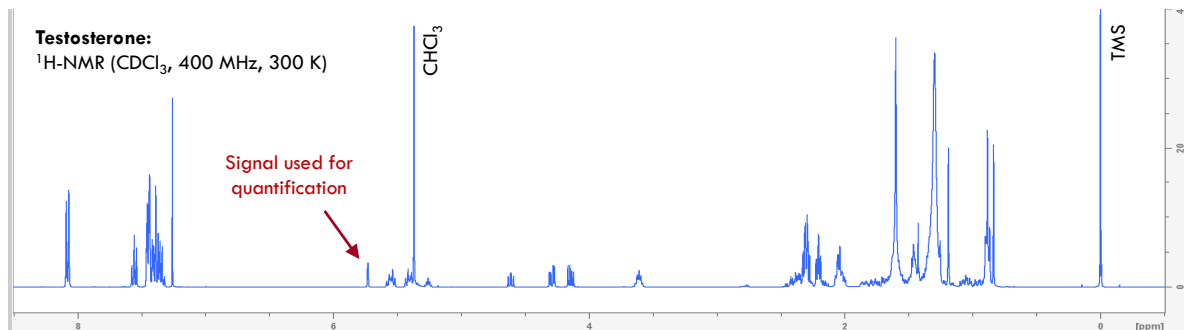
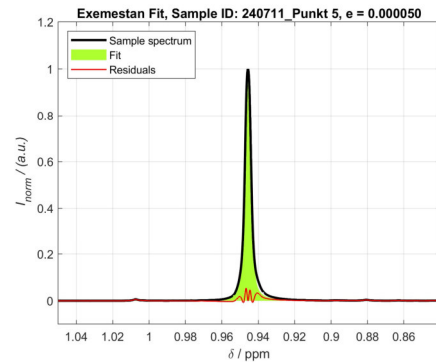
Steroids contain an anabolic moiety responsible for the accumulation of proteins in muscles and bones and an androgenic moiety responsible for the development of sexual characters.^[1] They are used by bodybuilders to boost muscle growth and performance. Prohormones enhance the natural synthesis of steroids. Anti-estrogens are used to minimize the side effects of anabolic steroids. All of these substances can cause cardiovascular diseases, liver and kidney issues, depression and addiction.^[2] In Germany, they are considered as medical substances, so their production and distribution are regulated by laws.^[3] Moreover, they are classified as doping substances by the Olympic Committee. That is why their study is a matter of concern.

MATERIALS AND METHODS

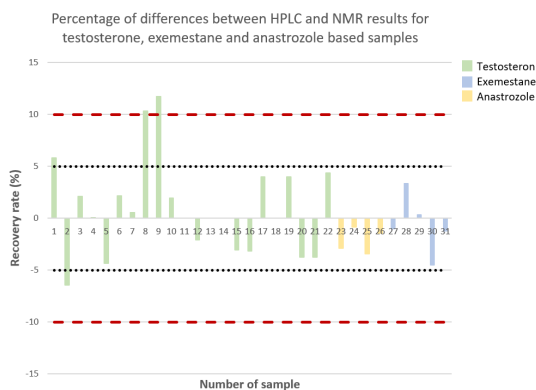
Sample Preparation

- 15 mg of injectable oil or 30 mg of milled tablet in a G4 vial
- 1 mL of (²H)Chloroform (vs. 50 mL of MeOH for HPLC)
- Shaking for 4 minutes (vs. stirring for 1.5 h for HPLC)
- Extraction with ultrasonication for 4 minutes
- Membrane filtration
- 600 µL into NMR tube → measurement

Automatic data evaluation and integration with Matlab



RESULTS



Substance	LOD (mg/g)	LOQ (mg/g)	Expected mass ratios w / (mg/g)	Obtained mass ratios w / (mg/g)	Regression Coefficient	Recovery rate (%)	Variation Coefficient
DHEA	3,95	9,51	18,8 – 170,5	15,88 – 159,9	0,9983	88,96	0,10
DHEA Acetate	3,65	8,86	11,4 – 171,5	9,5 – 160,0	0,9980	90,71	0,12
Testosterone	3,40	8,21	15,3 – 168,5	13,2 – 168,2	0,9990	95,86	0,10
Trenbolone	7,82	18,85	11,5 – 236,0	11,0 – 240,0	0,9975	100,21	0,08
Methandienone	2,08	5,00	15,5 – 79,5	14,0 – 80,0	0,9992	99,20	0,08
Anastrozole	1,01	2,38	3,4 – 20,5	3,4 – 22,0	0,9936	101,96	0,08
Exemestane	20,48	49,52	99,3 – 884,6	91,0 – 880,0	0,9988	96,12	0,06

CONCLUSION

This study has shown that NMR is more efficient and equally relevant as HPLC to determine anabolic steroids, prohormones and anti-estrogens in tablets or injection oils. The use of automatic data evaluation with Matlab as well as the no-need of reference substances up to date makes the new NMR method faster and easier to use than the previous HPLC method.

LITERATURE

- [1] Daniel Lednicher: *Steroid Chemistry at a Glance*, 2010, John Wiley & Sons, Ltd. ISBN: 978-0-470-66085-0
- [2] Horn: *Biochemie des Menschen*, 2020, Georg Thieme Verlag KG. ISBN 978-3-13-243342-7, pp 508-514
- [3] Gesetz über den Verkehr mit Arzneimitteln (Arzneimittelgesetz - AMG), https://www.gesetze-im-internet.de/amg_1976/;
Gesetz gegen Doping im Sport (Anti-Doping-Gesetz - AntiDopG), <https://www.gesetze-im-internet.de/antidopg/index.html>