Summary

Quantitative analysis of endogenous steroids (referred to as steroid profiling) is applied in doping analysis to detect the abuse of steroids that also have an endogenous origin, *e.g.* testosterone. The concentration of testosterone versus epitestosterone (T/E ratio) in urine is considered as the most sensitive parameter for detection of testosterone abuse. In Chapter 1 the validity of the T/E ratio is discussed by reviewing several factors that can influence this ratio, possibly leading to false-positive results. Despite these influential factors, the T/E ratio has been applied since the early eighties and can still not be displaced by the more recently developed isotope ratio mass spectrometry.

Chapter 2 describes an experiment in which the relation was investigated between the metabolism of alcohol and the increase of the T/E ratio. Male and female subjects consumed an average dose of alcohol (1.2 g/kg bodyweight). Urine and plasma samples were analyzed for testosterone, epitestosterone and for several precursors and metabolites of testosterone. All subjects showed an increase of the T/E ratio, during 10-12 hours after the start of consumption. However, the effect was significantly higher in females than in males. The chance of a T/E ratio increasing above the criterion of the International Olympic Committee (T/E = 6) by recreational alcohol consumption is realistic and should be taken into account, especially in out-of-competition doping control. Although the cause of T/E increase is not completely understood, simultaneous utilization of cofactors for both steroid biosynthesis and ethanol metabolism seems to play a predominant role.

Since steroid containing food supplements have become easily available, a frequently occurring abuse of steroids as dehydroepiandrosterone (DHEA) and androst-4-ene-3,17-dione (Δ4-AEDIONE) in sports is suspected. As described in Chapter 3, detection of these steroids in doping analysis is mostly performed by profiling techniques originally developed for detection of testosterone administration.

After oral administration of DHEA or Δ4-AEDIONE the T/E ratio and the excretion rate of metabolites as androsterone and etiocholanolone increases (Chapters 4-7). Profiling of these parameters can therefore not lead to specific information about the identity of the administered substance.

Chapters 4 and 5 describe excretion studies with respectively deuterium-labeled DHEA and Δ4-AEDIONE. Several oxygenated metabolites of these steroids were identified. The relative increase of the excretion rates after administration showed that the analysis of oxygenated steroids provides information of equal sensitivity as profiling the “regular” non-oxygenated metabolites.

Chapters 6 and 7 describe excretion studies with non-labeled DHEA and Δ4-AEDIONE. Urine samples were analyzed for oxygenated and non-oxygenated metabolites of both steroids. Sensitivity and specificity of each studied parameter were established. The most sensitive parameters for DHEA were 7β-hydroxy-DHEA, androst-5-ene-3β,17β-diol, 7-keto-androsterone, 6α/β-hydroxy-Δ4-AEDIONE, 16α-hydroxy-androsterone, 16α-hydroxy-etiocholanolone, androsterone, etiocholanolone and DHEA itself. High sensitivity for detection of Δ4-AEDIONE was observed for 4- and 6α/β-hydroxy-Δ4-AEDIONE, androsterone, etiocholanolone and testosterone. The most specific metabolites for DHEA (corresponding to a high increase in excretion rate after administration of DHEA and no or a low increase after administration of Δ4-AEDIONE) were 7β- and 16α-hydroxy-DHEA, 7-keto-DHEA, 7-keto-androsterone, androst-5-ene-3β,17β-diol and DHEA itself. The most specific metabolites for Δ4-AEDIONE were 4-hydroxy-Δ4-AEDIONE, testosterone, epitestosterone and 6α/β-hydroxy-testosterone. Concluding, the analysis of oxygenated metabolites can lead to additional information about the identity of the administered steroid, despite the relatively low conversion to these metabolites compared to the conversion to non-oxygenated metabolites.

Chapter 8 describes the derivatization of 3-keto-4-ene steroids. The trimethylsilylation of these steroids in a reagent of MSTFA/NH_4I/ethanethiol results in 3,5-dienolTMS derivatives. This is in contrast to the basic reagent MSTFA/KOAc/imidazole that mainly leads to the corresponding 2,4-dienolTMS isomers. An isomerization experiment proved that the 3,5-dienolTMS derivative of the model steroid 17-methoxy testosterone is more stable than the 2,4-dienolTMS isomer. The formation of thermodynamically least favorable 2,4-dienolTMS products was explained by hydrogen-deuterium exchange experiments. These showed that the protons at C^2 are more acidic than the protons at C^6. This leads to the formation of kinetically favorable 2,4-dienol products under the basic conditions of the MSTFA/KOAc/imidazole reagent.

Chapter 9 describes artifact formation that occurs during the derivatization of androsterone and etiocholanolone in MSTFA/NH_4I/ethanethiol. These artifacts were identified as ethyl thio-containing products of the respective trimethylsilyl derivatives. The conversion was significantly accelerated by addition of diethyl disulfide to the reagent prior to incubation. A mechanism is proposed for ethyl thio-incorporation at

the C^{16}-position. In this experiment the conversion to the artifacts was insufficient to significantly influence the analysis of androsterone and etiocholanolone. However, when the formation of new metabolites is investigated, the ethyl thio-incorporation can lead to false interpretations.