PREFACE

Steroid profiling is one of the established techniques in doping analysis for detecting the abuse of steroids that are identical to steroids of endogenous origin. It is based on the analysis of several steroids that can originate from the endocrine system, in urine samples. This analysis is mostly aiming at metabolites and/or precursors of testosterone.

Since the introduction of testosterone as a widely abused doping agent, the concentration ratio of testosterone versus epitestosterone (T/E ratio) has been considered as the most sensitive and robust parameter to consider for detection of testosterone abuse. Despite the fact that the T/E ratio is accepted as analytical method in doping analysis, it has often played a controversial role in doping cases. This is mainly caused by insufficient published data that is available about the parameters that could possibly affect the “natural” T/E ratio, resulting in a significant chance of false positive cases. Nevertheless, 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 for the detection of testosterone.

This thesis discusses several items that are related to the analytical chemistry of steroid profiling. In **part I** the main focus is placed on the T/E ratio. Chapter 1 discusses the possible limitations of the T/E ratio by reviewing potential influential factors on this parameter. One of those factors is the consumption of alcohol. In Chapter 2 an experiment is described in which the relation between the metabolism of alcohol and the increase of the T/E ratio is discussed. For this purpose male and female subjects consumed an average dose of alcohol.

Steroid profiling techniques used today have mostly been developed in the time that testosterone was one of the few abused compounds identical to endogenous steroids. T/E ratios higher than the cutoff criterion of six were regarded as specific evidence of testosterone abuse, unless a naturally high T/E ratio could be proven. However, in the present time the abuse of substances identical to endogenous steroids has become much more complicated by the introduction of precursors in the biosynthesis of testosterone as “food supplements“. An increased T/E ratio as a result of oral administration of steroids as dehydroepiandrosterone (DHEA) and androst-4-ene-3,17-dione has been described several times in literature. Therefore,

the T/E ratio should not be considered as a specific parameter for detection of testosterone abuse, but more as an indication that manipulation of the endocrine system has occurred.

Part II of this thesis is focussed on the development of steroid profiling techniques that provide better possibilities for identification of the steroids administered. Criteria of sensitivity (defined as the response of a parameter after administration) and specificity (the response of a parameter after administration of one particular steroid, compared to the response for other steroids) are used to evaluate new parameters in steroid profiling.

In Chapter 3 is described that detection of oxygenated metabolites could possibly provide information of equal sensitivity but higher specificity than non-oxygenated metabolites. In Chapters 4 to 7, aspects of sensitivity and specificity are investigated by performing excretion studies in male subjects with DHEA and androst-4-ene-3,17-dione used as model steroids. Additionally, an excretion study with 3-acetyl-7-keto-DHEA is evaluated in a case study design in an appendix.

In **part III** several analytical procedures of steroid profiling are discussed. As gas chromatography coupled to mass spectrometry (MS of MS/MS) is still considered as the method of choice for steroid analysis, derivatization is an essential sample preparation step to improve chromatographic behavior and to increase mass spectrometric sensitivity. Derivatization is often a bottleneck in the achieved analytical performance. In Chapter 8, trimethylsilylation reactions upon 3-keto-4-ene steroids were scaled up from an analytical (μg) to a preparative (mg) scale to gain fundamental insight into observed differences in product formation. The thermodynamically versus kinetically controlled formation of 3,5-dienolTMS and 2,4-dienolTMS derivatives is discussed.

Chapter 9 describes the formation of ethyl thio adducts with steroids upon derivatization with MSTFA/NH_4I/ethanethiol. These analytical artifacts could lead to interpretation problems in sample analysis.

In the final general discussion (Chapter 10) the perspectives of steroid profiling have been reviewed.