**Project Overview**

Since years high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) gained importance for the detection of various classes of doping relevant substances. In contrast to the classical GC-MS technique it allows for separation of analytes with different functional properties without derivatization.

However some analytes are still challenging as HPLC-MS/MS shows limited resolution capabilities and highly polar analytes interact only insufficiently on the conventional analytical columns. Thus, especially the HPLC analysis of several steroidal doping substances and their metabolites but also the polar stimulants are dissatisfactory. Supercritical fluid chromatography (SFC) as orthogonal separation technique to HPLC may help to overcome these issues.

During the project it will be tested for the analysis of stimulants. In this class some of the very polar compounds already proved to be nicely analyzed by SFC-MS/MS. A multi-analyte method will be developed and compared to the currently used method. Special focus is given to robustness, identification power and turn around times.

**Result and Conclusion:**

High performance liquid chromatography (HPLC) is considered as method of choice for the separation of various classes of drugs. However, some analytes are still challenging as HPLC shows limited resolution capabilities for highly polar analytes as they interact only insufficiently on conventional reversed phase (RP) columns. Especially in combination with mass spectrometric detection limitations apply for alterations of mobile phase composition and thereby also stationary phases. Some highly polar sympathetic drugs and their metabolites showed almost no retention on different reversed phase columns. Even on phenylhexyl phases, that show different selectivity due to \(\pi\)-\(\pi\)-interaction their retention remains poor.

Supercritical fluid chromatography (SFC) as orthogonal separation technique to HPLC may help to overcome these issues. Selected polar drugs and metabolites were analyzed utilizing SFC separation. In method development software assistance was used to create a robust separation. Thereby the response surface was generated as full central composite in quadratic design model focusing on the critical peak pairs, that show the same ion transitions in tandem mass spectrometry (MS/MS) and therefore require
chromatographic separation for proper assignment. In the final SFC-MS/MS method, all compounds showed sharp peaks and good retention also for the very polar analytes, such as sulfoconjugates. Retention times and elution orders in SFC are different to both RP and HILIC separations due to the orthogonality. Furthermore, short cycle times could be realized. As temperature and pressure strongly influence the polarity of supercritical fluids, a precise temperature and backpressure regulation is required for the stability of the retention times. As CO2 is the main constituent of the mobile phase in SFC solvent consumption and solvent waste are considerably reduced.