"Stable Isotope Analysis of Alkaloids and other biogenic Amines relevant to Doping Control"

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Project Summary

Stimulants generally belong to the substances prohibited in Sports. Among these there are several compounds that are easily available as over-the-counter drugs, that may be present in certain foodstuffs, or that may be produced in the human body from precursors in the diet. This gives rise to the possibility of accidental doping offenses on the one hand. But on the other hand, it also enables deceptive athletes to fraudulently relate a positive test result to dietary habits.

Hence, in case of a positive test for stimulants, it will be most advantageous to be able assign the relevant compounds to defined sources. Also the possibility to exclude certain sources will be most useful.

Problems like this can be addressed by stable isotope analysis. The elements hydrogen, carbon, nitrogen, and oxygen make up stimulants and related substances. These elements feature more than one isotope, atoms with slightly differing masses. The ratios of these isotopes depend on the primary source of the molecule. But they also may be changed during biosynthesis or degradation.

The project aims to exploit the information present in what is often called "isotope fingerprint". By stable isotope analysis we hope to be able to detect accidental application of prohibited substances as well as pretended pleas.

Results and Conclusions

- Methods have been developed for the 13C=12C and 15N=14N analysis of pure and urinary ephedra alkaloids.
- Comparably large amounts of the compounds are required especially for $^{15}N/^{14}N$ analysis. Valid $^{15}N/^{14}N$ analysis roughly requires 3 µg of any single ephedra alkaloid compound to be injected into the GC-C-IRMS system. In practice, this will be rarely achievable, in particular for the most interesting compounds NE and NPE when formed during metabolism.

- The methodology has been developed also to analyze synephrine and octopamine. Purity and in particular recovery are however far too poor for stable isotope analysis. This has therefore not been tested for synephrine and related compounds.
- $^{15}N/^{14}N$ analysis of ephedra alkaloids is fundamentally not suited for the purposes intended here. First of all, metabolism induces excessive nitrogen isotope fractionation. The fractionation is time dependent. After already short periods the 15N=14N ratios of the excreted drug will not correspond to that of the administered compound at all. In addition, there seems to be significant variation in the 15N=14N signatures of various drugs and pharmaceuticals. Eventually, no sufficiently definite 15N=14N isotopic link between drug and metabolite can be established. This would have to be evaluated much differently if valid 15N=14N analysis of ephedrine metabolites was possible.
- $^{15}N/^{14}N$ isotopic fractionation during metabolism of ephedra alkaloids appears to be inverse. This means that the parent compound becomes ^{15}N depleted. By contrast, "normal" isotope effects result in heavy isotope enrichment of the reagent because the isotopically lighter species tend to react faster. Inverse isotope effects have been rarely observed. This is therefore highly interesting in basic research but beyond the scope of the study.
- If at all, source assignment of urinary ephedra alkaloids can be based on $^{13}C/^{12}C$ analysis. Fractionation of carbon isotopes appears comparably small. In addition $^{13}C/^{12}C$ analysis in practice is much less demanding. In respect to ephedra alkaloids and similar compounds the amount required for valid analysis is ca. 20 less as compared to $^{15}N/^{14}N$ analysis.
- Combined $^{15}N/^{14}N$ and $^{13}C/^{12}C$ isotope fingerprinting appears promising for source assignment of unmetabolized ephedrine pharmaceuticals and preparations. This, however, is beyond the scope of the study.