SUMMARY

In recent years numerous cases of intoxication with plant alkaloids have been reported in Slovenia. Most of them were caused by unintended ingestion of autumn crocus, food containing buckwheat contaminated with alkaloids from the thorn-apple and voluntary usage of iboga bush root bark with the intent of ending the drug addiction. These events have motivated me to research options for the qualitative and quantitative determination of the most prominent and problematic alkaloids – atropine, scopolamine, colchicine, ibogaine and its metabolite noribogaine – in biological samples. I report that it is possible to identify all of the above mentioned substances in extracts of bodily fluids with routine GC-MS methods (capillary HP-5MS column). Since atropine and scopolamine are thermally degradable, I have studied their temperature related degradation and determined that most of the degradation can be avoided by sufficiently reducing injector temperature. Additionally, all five substances can be successfully identified with LC-MS/MS method on Zorbax XDB-C8 and Zorbax XDB-CN columns. The cyano column was then used to develop quantitative methods. Since all five substances are unlikely to occur in biological samples at the same time, I have developed three separate optimized and validated methods for their determination. The development of these methods was conducted in accordance with internationally accepted guidelines and consisted of several optimizations (MS/MS performance, chromatographic separation, volume and composition of injected solution), checking of selectivity and linearity, the choice of a calibration model, the determination of repeatability and accuracy (inter-daily and intra-daily), the determination of LLOQ and LOD. The methods have been validated on blood samples (plasma, serum) in the range of therapeutic and toxic concentrations of alkaloids, namely 0.10 to 50 ng/mL for tropanes (IS levobupivacaine), 0.50 to 100 ng/mL for colchicine (IS prazepam) and 0.25 to 100 ng/mL for ibogaine and noribogaine (IS prazepam); the lowest calibrant is also the LLOQ. All three methods fulfill all the required repeatability (RSD < 15 % and RSD < 20 % at LLOQ) and accuracy criteria (deviation ± 20 % from accurate value) for all alkaloids despite the short analysis time of 7 min. A LLOQ comparable to those reported in literature was achieved at a small sample consumption (100 to 170 μL) and using only simple deproteinisation. No other methods using only simple deproteinisation for the determination of scopolamine, colhicine and ibogaine levels in blood samples were found in the literature. During method validation the process efficiency (PE) and the diverse matrix effects (ME) were carefully studied. For the latter, I have introduced a new, modified approach and verified its validity by experiment. By exposing the samples to both visible and UV light I have determined that samples containing colchicine and ibogaine with metabolite must be protected from light (immediately after withdrawal) to avoid degradation. However, the compounds are stable enough that preparation of samples is possible even at normal light, if the aforementioned fast deproteinisation is used. All the substances are sufficiently stable to be safely stored short-term (several days at 4 °C) or long-term (up to a year at -20 °C).

Doctoral thesis presents the development of fast, sensitive and robust methods, which require a minimal quantity of sample and a minimal sample preparation. This goal was achieved by making the most of all the options and capabilities offered by the LC-MS/MS instrument. The methods described in this work have been successfully applied for the analysis of over 600 samples.

Keywords: alkaloids, biological samples, validated quantitative method, LC-MS/MS, GC-MS