

## Abstract

In recent years, there has been a growing interest in psychoactive substances, in particular legal highs, which are often a legal alternative to alcohol or classic drugs. Widely available, sold as bath salts or other preparations marked as not for consumption. Synthesized in home laboratories, they do not have a clearly marked composition, which only confirms the huge risk to health and even life these agents pose. The emerging new structures pose a huge challenge for toxicology laboratories and law enforcement agencies. The purpose of this doctoral dissertation was to develop and implement analytical methods for routine analyzes that allow the determination of the largest possible number of psychoactive substances, drugs and their metabolites in the shortest possible time in biological matrices - blood, urine and hair using liquid chromatography coupled with mass spectrometry. A very important element of the research was the development of an analytical method that would allow it to be easily adapted to a different matrix, and could be further developed by adding more analytes to be able to follow the expectations of the psychoactive substances market. The analytical methods developed as part of the research allowed for the simultaneous analysis of over 500 analytes during half an hour of analysis using LC-MS/MS.

Determination of psychoactive substances, drugs and their metabolites in biological material is a huge analytical challenge. The use of liquid chromatography coupled with tandem mass spectrometry gives great analytical possibilities to develop such a complex method, but still the analysis of such a number of substances during one analysis is a huge analytical challenge. The NPS analysis is associated with chromatographic difficulties due to the large number of isomers or compounds with a very similar structure. Each of the NPS groups contains structurally similar analytes, moreover, in the case of cathinones, we often encounter isomers and slight structural modifications that do not change the mass of the chemical compound, but are no longer prohibited substances by law. These facts show how difficult it is to achieve chromatographic separation of such a large number of analytes. Thanks to the use of the MRM pair tracking method, the properly selected conditions of the mass spectrometer allowed for the unambiguous identification of the analytes tested.

As a sample preparation method, liquid-liquid extraction was used, which allowed the isolation of the tested analytes belonging to different groups among NPS, which was not possible using solid phase extraction. The use of the standard curve in the matrix for quantitative research allowed to take into account the influence of the matrix on the results obtained for individual analytes and matrices. In addition, the final dilution of the samples instead of the

usual concentration allowed to reduce the influence of the matrix on the obtained results. The developed analytical methods allowed for the verification of samples of various matrices from one patient, which gives the opportunity to study the metabolism of NPS and to conduct retrospective studies.

The developed analytical methods were verified in proficiency tests, then implemented in routine analyzes of the Institute of Forensic Genetics in Bydgoszcz and accredited by the Polish Center for Accreditation. These methods have also been included in the patent application.