Paradigm shift in analyzing commonly abused drugs from biological matrices

Concept of the research

The two-decade data of drug abuse issues objectively mirror the experiences of the period of political, social and economical transition beginning after the changes of the Hungarian regime in 1989. The objective analysis and interpretation of these data and thus the judgement of the situation of drug abuse in Hungary is a highly complex issue. One of the numerous challanges results from the fact that the available data had no interpretable context, basic values were missing or were of uncertain reliability, surrounded by a constantly changing context. No normal value or range could be defined for the comparison of measurment data, hindering us from drawing conclusions about the high or law degree of national drug abuse.

The current interpretation of the available data and thus an objective judgement of the position of drug abuse in Hungary was facilitated by the *temporal and geographic* characterization of the available information, giving a picture of how serious the problematics of drug abuse in Hungary are in relation to other countries and allowing us to evaluate whether the trends of the past two decades can be considered a general tendency.

The last decade of the 20th century made our country play a *significant* role in struggling against illegal drugs both in terms of prevention and in establishing directive norms, as well as in elaborating standard analytical methods and their technical bases. With full knowledge of all these facts my PhD thesis makes a directive step towards adequatly answering the plethora of questins raised by illegal drug use in the 21st century.

My permanent activity as an expert of this field raised questions for which the research for experimental-based answers proved to be time-consuming and demanded a coordinated interdisciplinary approach.

Aims of the research

In my PhD thesis I aimed to answer the following questions:

1. To what extent the solid phase extraction [SPE] of the drug-containing matrix can fulfil the important requirement of forensic toxicology to minimize the amount of human biological sample used? What is the precise role of solid phase microextraction [SPME] in the qualitative and quantitative analysis of amfetamin derivatives?

2. How can drug use be monitored in rodent animal models? Are these animal models suitable for verifying the presumed harmful effect of cannabis derivatives on the lung, as suggested by recent anecdotal observations?

3. Can the experimental results be translated into the sampling methods of commonly abused drugs and psychotropic substances in case of both standard and yet to be standardized biological matrices?

4. Following the recommendations of the European Union how should the pathological sampling kit be modernized to allow for a standard sampling for the purposes of post mortem toxicological analysis and histological evaluation?

5. To what extent can the sampling and analytical testing of saliva, an approach not widely used in Hungary but internationally becoming increasingly widespread according to the literature, be introduced into rutin practice in our country? What are the methodological requirements to elaborate about saliva sampling and methods of its analytical testing?

6. What sort of practical experiences can the integrated system of extraction and analysis [TOX.I.S.], uniquely operating at the Department of Forensic Medicine and at the Department of Laboratory Medicine of the University of Pécs, offer for fighting against crime and preventing criminal events?

Executive summary of the research by chapters of the PhD thesis

The **first chapter** of my PhD thesis is a summary of the aims, scope and methodology of my research work.

Chapter 2 describes several toxicological definitions, presents the definition and classification of toxins, and defines the prerequisites of intoxication.

Chapter 3 describes the definitions of commomly abused drugs and psychotropic substances, followed by a summary of the characteristics of developing a drug abuse lifestyle within the Hungarian population. I give a description of the prerequisites and circumstances characterizing the era of the economical, political and social transition following the change of the socialist regime that 20 years ago played a role in Hungary's becoming a transit and subsequently a target country for illegal drug trade with an illegal (black) market for drugs

and a growing population of illegal drug users. I characterized the drug-addict personality from two different points of view: as someone whose deviant lifestyle inherently suggests the risk of criminal actions and someone who is a victim of the abuse potential of a chemical substance.

Chapter 4 deals with the preventive actions the society takes in order to suppress drug abuse. On the one hand policing organisations (eg. police and the Hungarian Customs and Finance Guard) take significant efforts and the law-maker body takes increasingly rigorous actions to suppress the supply side, while on the other hand efforts are made for drug prevention as a protective action for the demand side against developing a self-destuctive lifestyle.

Chapter 5 comprises the statistical analysis and a detailed description of my research work in terms of standardized sample-taking from living and deceased persons prosecuted for drug issues. I defined the requirements of official sample-taking, including the circumstances of the sample taking process, as well as the requirements for laboratories performing forensic sample analysis. Besides describing the standardized analysis of human samples (blood, urine) I *highlighted the need for a paradigm shift*. Mainly for the official evaluation of drug effect and based on a modern approach we should use a human biological matrix for the analysis. Sample-taking should be a simple, fast, hygienic, reliable (excluding the possibility of falsification) and non-invasive method, assuring a sufficient amount of sample from the donor. After studying various matrices I elaborated the methodology for official sample-taking of saliva, for which drug concentration correlates with serum concentration of the substance in question.

Chapter 6 describes the forensic toxicological analysis of several substances according to the methods I developed. Besides the analyses of morphine and cocain I describe two specific cases: an in vivo and a post mortem example relating to cocain abuse. The review of the issues about morphine includes the description of a pathological kit that can standardize post mortem sample taking according to the EU standards. The review of the issues about cocain includes the description of the analytical method (TOX.I.S.) that, for the first time in Hungary, I used during the crime investigation. I successfully adopted solid phase extraction (SPE) techniques for the analysis of morphine, cocaine and even cannabinoids. SPE has the advantage of requiring a minimal sample quantity and offering appropriate purity for the purpose of instrumental analysis, as well as having an excellent test–retest reliability.

Utilizing the volatile property of amphetamine and derivatives I elaborated a solid phase microextraction (SPME) technique and adopted for the routine analyses I executed.

Chapter 7 describes the animal experiments I performed. These experiments provided strong evidence that marihuana use produces a dramatic change in respiratory capacity. These changes proved to be extremely rapid even with regard to the amount of marihuana smoke inhaled.

Chapters 8–10 give a summary of my research work and the conclusions that can be drawn from the results. As a key point of my PhD thesis I answered the questions raised in Chapter 1, thus revealing my novel scientific results and theses. Finally I summarized my recommendations.

Novel scientific results – theses of my research

1. My research has provided evidence that compared to the tradicional methods of extraction SPE is more appropriate for minimizing the sample used and optimizing the analysis itself. For the first time in Hungary I have successfully demonstrated that solid phase microextraction (SPME) is prominently suiatble for the qualitative and quantitative analysis of amphethamine derivatives from a biological matrix.

2. My experiments in a murine modell have successfully demonstrated that urine analysis is suitable for measuring cannabis concentration and this value strongly correlates with the damage of lung function caused by marihuana. Based on my observations the lung-damaging effect of cannabis is no longer a theoretical assumption.

3. Based on my experimental research I published a Methodology Letter (OITI, ML. No. 1. 1999) for policing organisations regarding the analysis of commonly abused drugs and psychotropic substances. Ten years after their publication these recommendations are still operative.

4. In line with the recommendations of the European Union I have developed a standardized kit for the purpose of forensic pathological analysis. Using this kit definitely improves the accuracy and efficiency of post mortem toxicological analysis and pathological examinations.

5. Taking international data into consideration I have elaborated the precise methodology for taking saliva samples. I have successfully demonstrated that on-the-scene sample-taking is a safe and hygienic method providing high accuracy via its timely execution. Compared with other matrices in use this biological sample theoretically provides a better chance to determine the actual degree of drug effect.

6. For the first time in Hungary I have employed a TOX.I.S. analytical system for the investigation of collective cocain abuse. The integrated approach offered a fast and efficient way of discovery contributing to deterrence.