1. Introduction

In forensic toxicology analytical challenges are daily routine. For the presented work showcase analytes have been chosen covering some parts of the broad spectrum in forensic toxicology. The work focused on the role of LC-MS/MS in forensic toxicology for answering questions in forensic toxicology as well as in therapeutic drug monitoring. As biological matrices blood plasma and hair, as alternative matrix, were chosen.

The first group of medicaments that was investigated in this work was the “Viagra” group. These life-style medicaments are often faked and traded on the black market. They are inhibitors of the human cyclic guanosine monophosphate–specific phosphodiesterase type 5 enzyme (PDE-5). Sildenafil (Viagra, Revatio), vardenafil (Levitra), and tadalafil (Cialis, Adcirca) were investigated. Typical indication for these medicaments is oral treatment of erectile dysfunction [1]. But, due to the fact that PDE-5 is also present in lung blood vessels, its inhibition leads to selective pulmonary vasodilatation. Therefore, sildenafil and tadalafil are also licensed for the treatment of pulmonary arterial hypertension [2]. Thus, this group is not only of forensic interest, but is also topic in therapeutic drug monitoring.

Medicaments can have a high influence on the fitness to drive a car, especially in combination with alcohol. That is the fact for the group of the benzodiazepines and the so-called z-drugs – they were the second group of substances investigated in this work. They are prescribed as tranquilizers, hypnotics, anticonvulsants or muscle relaxants. They have a high potential of addiction and can lead to life-threatening intoxication [3-11]. The z-drugs, namely zolpidem, zopiclone and zaleplon, do not have the same chemical structure as the benzodiazepines, but they are also agonists at the benzodiazepine receptor.

Due to the constantly changing drug market [12-13], it is important to be up to date. Next to the classic drugs of abuse, new psychoactive substances play a big role. The cathinones including beta-keto-amphetamines (methylone, butylone, ethylone), methcathinone and mephedrone as well as the piperazines (such as benzylpiperazine, metachloro-phenylpiperazine and trifluoromethylpiperazine) are often sold via internet as ‘bath salts’ or ‘plant food’. Little is known about the pharmacological and toxicological effects of these substances, some studies have been published recently [14-21] but still not answering all relevant questions about pharmacology and toxicology of these substances. Due to the facts that new psychoactive substances do not interact with the common immunoassays and that not all laboratories test for them, there is only little information about the actual prevalence of these substances.
2. Aims and Scopes

The aims of the presented studies were: 1. Development and validation of a quantitative method for the determination of phosphodiesterase-5 inhibitors and two of their metabolites in blood plasma with an application to therapeutic drug monitoring (TDM) and forensic cases, 2. Development and validation of a quantitative method for the determination of benzodiazepines and z-drugs in hair by using the MRM-EPI mode and application to driving ability assessments, and 3. Extension of the classical drug screen by developing a qualitative screening method for new psychoactive substances in hair and application to retrospective studies for assessment of prevalence.

3. Results and Discussion

Study 1: A LC-MS/MS method was developed for quantification of the PDE-5 inhibitors sildenafil, vardenafil, and tadalafil and 2 of their normetabolites (norsildenafil and norvardenafil) in blood plasma after a liquid-liquid extraction. The assay was validated according to international guidelines [22-23] and all analytes fulfilled the required criteria. For details see [24]. Selectivity was given for the tested compounds. Linearity ranged from 5 to 1000 ng/mL for sildenafil, from 2 to 700 ng/mL for norsildenafil, from 0.5 to 350 ng/mL for vardenafil, from 0.5 to 200 ng/mL for norvardenafil, and from 5 to 1000 ng/mL for tadalafil. Matrix effects were in acceptable ranges, as well as accuracy, repeatability, and intermediate precision. Repeated freezing and thawing or processing samples did not lead to any instability. The method was applied to 2 forensic cases. Tadalafil in the concentration of 360 ng/mL could be determined in the blood of a 79-year-old man, who died after sexual intercourse. In the second case, a 39-year-old man was guilty of rape. He admitted the ingestion of tadalafil before crime to enhance his performance. His blood concentration of tadalafil was 150 ng/mL. In order to prove the applicability in therapeutic drug monitoring, 46 patients, who were treated with sildenafil because of their pulmonary arterial hypertension, gave blood for analysis. Not only the concentration of sildenafil was used for therapeutic drug monitoring; the concentration of the main metabolite, norsildenafil, was monitored too. The determined concentrations of sildenafil ranged from 5 to 312 ng/mL, for norsildenafil from 4-319 ng/mL (see Figure 1). The large bandwidth of these results could be caused by different effects. Patients with pulmonary arterial hypertension often have other severe diseases, e.g. liver diseases or impaired renal function, leading to altered pharmacokinetics. Also, they often have to take many medicaments which could interact with sildenafil metabolism.

![Fig. 1. Concentration of sildenafil and norsildenafil in 46 PAH-Patients.](image-url)
Study 2: A LC-MS/MS method was developed for quantification of 21 benzodiazepines and three z-drugs in hair. A two-step extraction procedure was optimized. For pulverisation of hair a tungsten carbide ball in an Eppendorf tube shaken by a benchtop shaker found to be very effective. The following two extraction steps took place in the same disposable Eppendorf tube, that minimized any carry-over effects. The analytes were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) (electrospray ionization; multiple-reaction monitoring mode – information dependent acquisition – enhanced product ion scan (MRM-IDA-EPI)). For details see [25]. Selectivity was given for the tested compounds (alprazolam, 7-aminoclonazepam, 7-aminoflutrazepam, bromazepam, chlordiazepoxide, clonazepam, N-desalkylflurazepam, diazepam, flunitrazepam, flurazepam, alpha-hydroxymidazolam, lorazepam, lormetazepam, midazolam, nitrazepam, nordazepam, oxazepam, phenaazepam, prazepam, temazepam, triazolam, zaleplon, zolpidem and zopiclone). All compounds fulfilled the validation criteria according to international guidelines, except bromazepam. For an unambiguous identification the MRM-IDA-EPI mode was used. The resulting EPI spectra were compared to an in-house library. The method was applied to driving ability assessment cases. Several hundred cases had been analyzed. In nearly 25 % of the positive cases, zolpidem was tested positive, followed by nordazepam und lorazepam (see Figure 2).

Study 3: A LC-MS/MS method was developed for screening for new psychoactive substances in hair. For details see [26]. Routine extracts from hair samples that were tested positive for amphetamines or MDMA (samples of 2009 and 2010, N= 325) were reanalyzed for new psychoactive substances such as cathinones (methylone, butylone, ethylone, MDPV, mephedrone (4-MMC), methcathinone and cathinone), pipерazines (BZP, mCPP and TFMPP), 4-fluoroamphetamine (4-FA), methylphenidate (MPH) and ketamine (KET). Routine protocol for extraction of drugs of abuse involved a two-step extraction procedure with methanol and methanol/hydrochloric acid. The analytes were analyzed using LC-MS/MS in the MRM-IDA-EPI mode. The resulting EPI spectra were compared to an in-house library. In 37% (120 cases) of the analyzed cases new psychoactive substances could be detected. 14% (45 cases) of the cases were tested positive for ketamine. The pipерazine mCPP could be detected in 10.5% (34 cases) of the cases, five of these cases were also tested positive for trazodone, an antidepressant that is metabolized to mCPP. TFMPP was positive in one case, as well as methylone. 5% (16 cases) of the investigated cases were tested positive for methylphenidate and in 4% (12 cases) 4-Fluoroamphetamine could be detected. Mephedrone was identified in 3% (11 cases) of the analyzed cases (see Figure 3). This retrospective study proved that these new psy-
Psychoactive substances are consumed and that their prevalence is high. Clinical and forensic toxicological laboratories should include the most common ones in their daily routine.

In the presented work, LC-MS/MS has successfully been used for tackling particular problems in forensic toxicology. Validated quantification of different classes of drugs in blood as well as in hair and even screening for new psychoactive substances in hair were possible employing this versatile technique emphasizing the role of LC-MS in forensic toxicology.

4. Acknowledgements

I would like to thank the GTFCh for providing the travel fund for my presentation at the 2011 SOFT-TIAFT Meeting in San Francisco (CA).

5. References

[12] Bovens M, Schlaepfer M. Designer drugs/ research chemicals/ legal highs - a survey of recent seizures and an attempt to a more effective handling from a Swiss perspective. Toxichem Krimtech 2011;78:167-175.