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Quantitative analysis of new generation antidepressants using gas chromatography-mass spectrometry. Applications in clinical and forensic toxicology

Introduction: Depression is a chronic or recurrent mood disorder that affects economic and social functions of about 121 million people worldwide, and can eventually lead to suicidal behaviour. According to the World Health Organisation, depression will be the second leading contributor to the global burden of disease, calculated for all ages and both sexes by the year 2020 [1]. Therefore, the prescription rate of antidepressants (ADs) will increase, resulting in a growing interest for determination methods in the clinical and forensic field. Detection and quantification of ADs in plasma is a valid tool to optimize AD pharmacotherapy for special patient populations and for monitoring patient compliance [2, 3]. Analytical methods for the detection of ADs in blood and tissues are of interest in the field of forensics as they are often involved in intoxications [4].

Nowadays, the 'new' generation ADs are the most prescribed antidepressant drugs in the seven major markets (Japan, USA, France, UK, Italy, Spain, Germany) [5]. The 'new' generations include the Selective Serotonin Reuptake Inhibitors, the Selective Noradrenalin Reuptake Inhibitors, the Serotonin and Noradrenalin Reuptake Inhibitors, the Noradrenergic and Specific Serotonergic ADs, and the Serotonin-2 antagonists and Reuptake inhibitors [6, 7]. The selected ADs (mirtazapine, viloxazine, venlafaxine, trazodone, citalopram, mianserin, reboxetine, fluoxetine, fluvoxamine, sertraline, maprotiline, melitracen, paroxetine) are monitored in combination with their (active) metabolites as the latter can also contribute to the overall therapeutic and toxic effects. In addition, metabolites can give extra information about the time of ingestion, the metabolic capacity, and compliance. These metabolites, i.e. desmethylmirtazapine, O-desmethylvenlafaxine, m-chlorophenylpiperazine, desmethylcitalopram, didesmethylcitalopram, desmethylmianserin, desmethylfluoxetine, desmethylsertraline, desmethylmaprotiline, were chosen according to the AGNP-TDM expert group consensus guidelines [8].

Objective: The aim of this Ph.D. project is to develop and validate a gas chromatographic-mass spectrometric method (GC-MS) for the new generation ADs and their metabolites in plasma for therapeutic drug monitoring purposes. During the validation, a comparison between electron (EI) and chemical ionization modes (CI) was made. Moreover, this method was adapted and re-evaluated for post-mortem blood, brain tissue and hair for forensic investigations.

Results and discussion: A GC-MS method for the simultaneous determination of the 'new' ADs and their metabolites is validated in plasma, blood, brain tissue and hair using different ionization modes [9]. Sample preparation consisted of a strong cation exchange mechanism and derivatisation with heptafluorobutyrylimidazole [10]. The GC separation was performed in 24.8 minutes. Identification and quantification were based on selected ion monitoring in electron and chemical ionization modes. Calibration by linear and quadratic regression for EI and CI, respectively, utilized deuterated internal standards and a weighing factor $1/x^2$. Limits of quantification (LOQ) were established between 5-12.5 ng/ml in EI and positive ionization chemical ionization (PICl), and 1-2.5 ng/ml in negative ionization

chemical ionization (NICI) for plasma. For blood the LOQ ranged from 5-20 ng/ml, while in brain tissue it ranged from 25-62.5 ng/ml in PICI. During validation stability, sensitivity, precision, accuracy, recovery, and selectivity were evaluated for each ionization mode and were demonstrated to be acceptable for most compounds. While it is clear that not all compounds can be quantified either due to irreproducible validation results and chromatographic problems (trazodone) or due to derivatisation problems (O-desmethyl-venlafaxine), this method can quantify most ADs in the therapeutic range in plasma, in blood and brain tissue.

EI is the traditional method for comprehensive screening procedures due to the easy library search mechanism. This ionization, however, leads to high fragmentation of citalopram, melitracen, venlafaxine, and O-desmethylvenlafaxine, resulting in the aspecific high abundance quantifier ion at m/z 58 and inherent loss of specificity, especially for demanding matrices such as brain tissue. CI is a 'softer' ionization technique, providing more selectivity through molecular mass information. However, due to less fragmentation, the qualifier ions had low abundance, leading to loss of sensitivity. NICI leads to improved sensitivity due to heptafluorobutyryl-derivatisation, allowing a minimized sample volume. On the other hand, underivatized tertiary amines such as citalopram, melitracen, mianserin, and mirtazapine are not detected. CI modes can surely provide advantages, but the system is less robust and harder to optimize. The presence of impurities in the reagent gas, radical species in the ion source plasma (formed by trace amounts of oxygen, water or chlorinated solvents), air leaks and interactions with the ion source walls can lead to variations in spectra and thus difficulties during analysis. In addition, in routine clinical analysis, changing the EI and CI source can be time consuming. Therefore, EI is still the ionization mode of choice in clinical analysis. For routine toxicological analyses, PICI mode can be of interest when highly fragmented compounds have to be monitored. The NICI-mode leads to remarkable high sensitivity for the derivatized ADs and is therefore very interesting in paediatric clinical analysis where often only a limited amount of sample is available.

Future perspective: Several post-mortem cases were already successfully analysed with this SPE-GC-MS method. In the near future, patient plasma samples will be analysed to prove the method's usefulness in clinical settings. The TDM results will be related to the patient's CYP 2D6 profile to observe the relationship between metabolism, plasma concentrations and effect.

References

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