K30 Development and Validation of a Method Using Gas Chromatography/Mass Spectrometry (GC/MS) After Liquid-Liquid Extraction (LLE) for the Detection and Quantification of Clotiapine in Blood and Urine and Its Application to a Postmortem Case

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The goal of this presentation is to inform attendees of possibly fatal complications due to clotiapine intake by considering the autopsy results and the toxicological findings, including substance concentration and distribution in biological samples.

This presentation will impact the forensic science community by providing a reliable method for the detection and quantification of clotiapine in blood and urine samples by means of GC/MS after LLE.

Introduction and Goals: Clotiapine is an atypical antipsychotic of the dibenzothiazepine chemical class. It was first introduced in a few European countries in 1970. Despite the high incidence of extrapyramidal side effects, it has demonstrated efficacy in treatment-resistant schizophrenic patients.

Here, a method for the detection and quantification of clotiapine in blood and urine samples by GC/MS after LLE has been developed and fully validated. The method has been applied to a fatal case involving a 45-year-old man found dead in his private apartment.

Methods: For the extraction, blood, urine, and gastric contents were extracted according to the following procedures: to 1mL of each liquid sample were added 1mLl of deionized water and 500ng of methadone-d9 as internal standard. Samples were extracted at pH 8-8.5 (50mg of solid HCO_3-/CO_3^{2-}-buffer added) with 4mL of extraction solution (n-hexane/dichloromethane (85/15v/v) for 15min. After centrifugation (4,000 rpm, 3min) the organic layer was evaporated to dryness under nitrogen flow. The residue was reconstituted with 50µL of ethyl acetate.

GC analysis was carried out on an Agilent® HP 7028A GC coupled with an Agilent® MSD 5975. The capillary column used was an HP-5MS (17m x 0.25mm I.D. coated with a 0.25µm film). The GC conditions were as follows: the column temperature was programmed from 120°C to 290°C with an increase of 10°C/min; the injection port and the transfer line temperature was 270°C; helium was used as carrier gas with flow rate of 1mL/min; the split injection mode had a ratio of 15:1. The mass analyzer operated by electron impact (-70eV) in the Selected Ion Monitoring (SIM) mode. Quantitative analysis was carried out recording ions m/z 209-244-343 for clotiapine and m/z 78-165-303 for methadone-d9. The underlined ions were used for quantitative analysis.[1]

Application to a Postmortem Case: A 45-year-old man died in bed in his private apartment. Relatives could offer very little information about the circumstances of death, but positive information about mental illness (schizophrenia) was reported. The autopsy showed the presence of bladder over-distension due to massive urinary retention and contained 3.7L of urine. All other organs were unremarkable, with only a moderate pulmonary edema being found. The toxicological analysis, using the method developed above and validated showed the following concentrations: 1,318ng/mL in peripheral blood, 487ng/mL in urine (the clotiapine concentrations in urine samples were creatinine-normalized) and 1,860ng/mL in the gastric contents. No other drugs nor alcohol were detected in the biological samples.

Conclusions: A reliable method for the detection and quantification of clotiapine in blood and urine samples has been developed and validated. The application of the method to the reported case allowed identification of clotiapine at very high concentrations in blood, urine, and gastric contents, although there is little evidence in literature about its toxic values. In this case, the cause of death, taking into consideration the autopsy and toxicological findings, was due to a post-renal failure due to a severe bladder over-distention induced by clotiapine.

Reference:

1. Peters et al., Validation of New Methods, *Forensic Sci. Int*. Volume 165, 2007.

Clotiapine, GC/MS Method, Postmortem Case