

ILKKA OJANPERÄ<sup>1</sup>, MERJA GERGOV<sup>1</sup>, MILANA LIIV<sup>2</sup>, AIME RIIKOJA<sup>2</sup> and ERKKI VUORI<sup>1</sup>

<sup>1</sup> Department of Forensic Medicine, P.O. Box 40, FI-00014 University of Helsinki, Finland,

<sup>2</sup> Estonian Bureau of Forensic Medicine, Narva mnt 46, 10150 Tallinn, Estonia

Abuse of fentanyls is a problem in Russia, and since 2001 seizures have also taken place in countries close to the Russian border, such as Estonia, Finland, Lithuania, Sweden, and the Ukraine. 3-Methylfentanyl (TMF) has been seized in Finland and Estonia, and fatalities due to this substance have occurred especially in the latter country.

Illicit fentanyl analogues are potent opioids exhibiting low concentrations in biological samples, and consequently, they require dedicated analytical methods to be detected and quantified. Previously, analysis of such drugs in human samples has mainly been performed by urine and blood radioimmunoassay. This technique cannot, however, differentiate between various fentanyl analogues. By LC-MS/MS, we were now able to determine TMF concentrations in victims of fatal overdose. Based on GC-MS screening for TMF in diverse specimens and subsequent LC-MS/MS quantification, we describe the key figures of forty-eight TMF related fatalities in Estonia 2005.

The LC-MS/MS method covered 23 fentanyls and other opioid drugs. Blood and urine samples were extracted with butyl acetate at pH 7. LC was performed on a Gemini C18 column using an acetonitrile - ammonium acetate gradient at pH 3.2, with a total run time of 38 min. Mass spectrometric analysis was performed with an electrospray triple quadrupole instrument in positive mode, using multiple reaction monitoring (MRM). MS conditions were optimized individually for each compound, and detection was split into four time periods to ensure sufficient sensitivity. Quantification was performed using fentanyl-D5, morphine-D3, codeine-D3 or buprenorphine-D4 as an internal standard. The limit of quantification for all compounds was near or below the lowest known reference levels.

All the forty-eight cases that screened positive for TMF by GC-MS could be confirmed by LC-MS/MS. In blood, median cis-TMF, trans-TMF and fentanyl concentrations were 1.0, 0.6, and 8.1 µg/l, respectively. In urine, median cis-TMF, trans-TMF, fentanyl and norfentanyl concentrations were 1.2, 1.2, 12, and 20 µg/l, respectively. The majority (96%) of the victims was males; their median age was 26 years, ranging from 17 to 41 years. One of the two female victims was 24, the other 25 years. A needle mark was found in forty-six victims. Poisoning by TMF alone or in combination with other drugs was in most of the cases considered the underlying cause of death. Exceptions were drowning in a bath (two cases) and explosive trauma (one case).

The TMF blood concentrations presented in this study are based on a considerable number of cases, and consequently they form a solid basis for future postmortem evaluations regarding this substance. The concentrations found are significantly lower than the levels reported in the literature for alpha-methylfentanyl and fentanyl.

**KEYWORDS:** 3-methylfentanyl, Blood concentration, LC-MS/MS, Postmortem

**Corresponding author:** [ilkka.ojanpera@helsinki.fi](mailto:ilkka.ojanpera@helsinki.fi)