Detection of benzodiazepines in hair by ELISA and LC-ESI-MS-MS

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This study was designed to validate an enzyme linked immunosorbent assay (ELISA) and liquid chromatography-tandem mass spectrometry (LC-MS-MS) method for the detection of nine benzodiazepines in hair. Sixteen hair case samples were tested from drug-related deaths where a positive benzodiazepine blood result was obtained. The case samples were decontaminated with 0.1 % sodium dodecyl sulfate, distilled water and dichloromethane. For ELISA analysis, the samples were extracted by incubation in monobasic phosphate buffer for 1 hour and then neutralized with dibasic phosphate buffer. They were diluted 1:5 with phosphate buffer saline (PBS) prior to analysis.

For LC-MS-MS, the samples were incubated overnight in methanol/25 % ammonium hydroxide (20:1) and subsequently extracted by solid phase. Thirteen samples were confirmed positive by LC-MS-MS. The benzodiazepines detected included diazepam, nordiazepam, temazepam, oxazepam, nitrazepam and lorazepam. The respective limits of detection were 0.13, 0.24, 0.09, 0.11, 0.03 and 0.62 ng/30 mg by LC-ESI-MS-MS. Limits of quantitation were 0.22, 0.41, 0.16, 0.19 and 0.05 and 1.02 ng/30 mg. Using a cut-off concentration of 0.1 ng/mg oxazepam, the Immunalysis® Benzodiazepine Microplate ELISA demonstrated a sensitivity and specificity of 100 % and 81 % respectively, compared with LC-MS-MS results.

KEYWORDS: *Hair analysis, Benzodiazepines, ELISA, LC-ESI-MS-MS*

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