

Simultaneous quantification of four benzodiazepines from whole blood by high-performance liquid chromatography in forensic toxicological analysis

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Abstract A rapid high performance liquid chromatography method, using a monolithic column, was developed for quantitative determinations of benzodiazepines (diazepam, clonazepam, lorazepam, midazolam) in whole blood. A liquid-liquid extraction step with n-chlorobutane isolates the drugs from alkalized blood. The separation was carried out in reversed phase conditions using a Chromolith Performance (RP-18 100x4.6 mm) column. For the mobile phase, a mixture of a phosphate buffer (pH= 2.5)/acetonitrile (65/35 v/v), in isocratic mode at 2 mL/min. An ultraviolet spectrophotometer was used as the detector at the wavelength of 220 nm. The total run time of the analytical method is less than 4-6 minutes. The calibration curves showed linearity and the correlation coefficient of each individual curve was greater than 0.995. The method was linear over a concentration range of 0.03-0.6 µg/mL for clonazepam, lorazepam and midazolam. For diazepam of linearity was over the range 0.04-5.0µg/mL. Quantification limits ranged from 0.03-0.04µg/mL and the accuracy were from 80% to 105% for the recovery test.

The results indicate that this analytical method is simple, specific, accuracy, sensitive, demonstrating from the validation data and a higher robustness. The proposed method is applied routinely in forensic toxicological analysis involving blood.

Keywords: benzodiazepines, forensic toxicological analysis, HPLC, monolithic column
