

Quantification of basic lipophilic drugs in blood without reference standards by LC-CLND based on extraction recovery estimation

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Limited availability of primary reference standards for new and rare compounds, designer drugs, and metabolites severely hinders the analysis of these compounds by conventional techniques. Especially quantification has been impracticable. Liquid chromatography-chemiluminescence nitrogen detection (LC-CLND) has been previously used in combinatorial chemistry and in quantification of seized samples in forensic science. Because of CLND's equimolar response to nitrogen, it is a suitable detector for quantification using only a single secondary standard. When extraction is used in sample preparation for LC-CLND analysis, the establishment of extraction recovery is essential. In this study, liquid-liquid extraction recoveries for basic lipophilic drugs were determined in whole blood. Subsequently, autopsy blood samples and external proficiency testing blood samples were quantified without reference standards based on the estimated extraction recoveries.

METHODS: Recovery of extraction for more than twenty drugs was determined in 0.2 and 1.0 mg/L concentrations in four parallel analyses. Whole blood (5 mL) was extracted at pH 11 with 10 mL of butyl chloride-isopropanol (98+2), and the evaporated extract was reconstituted with 100 µL of methanol - 0.1% formic acid (1+1). LC separation was performed in gradient mode with a Phenomenex Gemini C-18(2) 150 × 2 mm (3 µm) column, mobile phase consisting of 0.1% formic acid and methanol. UV diode-array detector was used for identification and CLND for quantification. Caffeine was used in external calibration.

RESULTS: The average recovery of extraction for basic lipophilic compounds was 84% and RSD of parallel analyses was generally <10%. CLND analysis of autopsy case blood samples showed comparable results with those of an established gas chromatographic method, the difference between these methods being on average 30%, ranging from 5 to 54%. The difference between the CLND results and the consensus reference concentrations of proficiency test samples was on average 17%, ranging from 0 to 37%.

CONCLUSIONS: LC-CLND is a unique instrument for quantification of nitrogen containing substances, such as drugs, without reference substances. For biological samples extraction procedures are often necessary and the recovery must be estimated. The present study shows that such a procedure is accurate enough to be of value in postmortem forensic toxicology for cases in which reference substances are not available. However, extraction recovery is affected by several factors, such as quality of autopsy blood sample and chemical properties of the analyte in question.

KEYWORDS: *LC-CLND, Extraction recovery, Lipophilic drugs*

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