DETECTION AND QUANTITATIVE DETERMINATION OF ANTIDEPRESSANT CLOMIPRAMINE IN BIOLOGICAL FLUIDS

Tomarovska L. Yu., Bayurka S.V.
The National University of Pharmacy, Kharkiv, Ukraine
tomarovskaya1992@mail.ru

Antidepressant poisonings occupy a leading position among the psychotropic drug intoxications in the world. Clomipramine (3-chloro–10,11–dihydro-*N*,*N*-dimethyl–5*H*-dibenz[*b*,*f*]azepine–5–propanamine) is a tricyclic antidepressant which is widely used in the modern treatment of the depression disorders. However, the medicine is associated with neurological side effects including worsening depression, other mood symptoms, suicidal thoughts or attempts. Clomipramine was the primary cause of acute and lethal poisonings. The postmortem concentrations were reported: blood 0.21–4.9 mg/L, urine 0.35–25 mg/L. The aim of this study was to develop methods of clomipramine isolation from blood and urine with liquid-liquid procedure followed by the analysis of the extracts obtained with help of colour tests, TLC, UV-spectroscopy, extraction-spectrofotometric methods. Clomipramine was extracted from the biological fluids by chloroform from alkaline medium at pH 11. Concomitant admixtures were separated by extraction with diethyl ether from acidic medium at pH 1–2. When isolating clomipramine from blood previously the protein admixtures were separated by adding 10% trichloroacetic acid solution followed by centrifugation. Reaction with concentrated sulphuric acid, Marqui's test, Frede's test, Erdman's test, Mandelin's test, Liebermann's test were positive for clomipramine. TLC identification was performed using mobile phase: chloroform-dioxane-acetone-25 % ammonium hydroxide solution (47.5:45:5:2.5), Dragendorff spray was used as location reagent, Rf=0.70±0.02, the sensitivity is 1 μg of the drug in the sample extract. Identification of clomipramine isolated from the biological fluids was performed with the help of UV-spectroscopy after clean-up step by TLC method. The UV-spectrum of the methanol eluate containing clomipramine was identical to the spectrum of the standard methanol solution of the drug. The wavelength of the principal peak was 251±2 nm. Quantitative content of the drug in the extracts was determined by extraction-photometric method by the reaction with methyl orange, an acidic azodye (linearity was in the range of 5–90 μg in the sample and it was represented by the following regression equation: Y=0.0137X+0.09). Recovery (precision, **%**RSD) of the methods developed were 79% (4%) for urine and 34 (4%) for blood. The results of validation have proven that the methods developed are accurate, precise, sensitive and linear in the range of the expected content of clomipramine in the biological fluids in fatal cases.