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Determination of Tramadol in Human Plasma by High-Performance Liquid Chromatographic Method with Fluorescence Detection

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A validated fluorescence determination of tramadol in human plasma was developed. Human serum samples (1.0 mL) spiked with known concentration of tramadol and 6.6 μg atenolol as an internal standard were alkalinized with 200 $\mu\ell$ of 0.5 N NaOH and extracted with 7 mL of *tert*-butyl methyl ether for 5 min. Extracts were centrifuged and 6 mL of organic layer was backextracted with 200$\mu\ell$ of 0.1N HCl for 3 min. Thirty microliters of centrifuged aqueous layer were injected onto reversed-phase octadecyl column and eluted with a mixture of 0.1M KH_2PO_4, acetonitrile and triethylamine (70 : 30 : 0.1, v/v, adjusted to pH 5.9 by phosphoric acid) at a flow rate of 1.0 mL/min. Fluorescence detection was performed at 270 nm (excitation) and 300 nm (emission). The precision and accuracy was found to be satisfactory over the whole range tested (20 ~ 500 ng/ml). The calibration curve obtained using peak area ratios showed a good linearity (r^2 = 1 in the concentration range 20 ~ 500 ng/mL in plasma). The recovery of tramadol was 98% at the concentration of 50 ng/mL. This method proved to be readily applicable to the assay of tramadol in human plasma samples.