HPLC with fluorescence detection of morphine in rat plasma using 4-(4,5-diphenyl-1H-imidazol-2-yl)benzoyl chloride as a label

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A highly sensitive method has been developed for determination of morphine (MOR) in rat plasma by using derivatization with 4-(4,5-diphenyl-1H-imidazol-2-yl)benzoyl chloride (DIB-Cl), a solid-phase extraction, a reversed phase column and a fluorescence detection. Cyclazocine was used as an internal standard. Derivatization could be achieved under mild conditions (e.g., 10 min at room temperature). To remove excess DIB-Cl, a solid-phase extraction with an ODS cartridge was used and the obtained recovery for MOR spiked to plasma sample was 92% (n=4). DIB-MOR was separated within 40 min by an ODS column and a step-wise elution program with acetonitrile and 0.1 M acetate buffer (pH 5.4). Fluorescence of DIB-MOR at 486 nm with excitation at 355 nm was monitored. Calibration curve of MOR spiked to rat plasma sample showed a good linearity (r=0.998). The detection limit of MOR in rat plasma at signal-to-noise ratio of 3 was very low (0.09 ng/ml). Both intra- and inter-day precision of the proposed method was also satisfactory (less than 9.4% RSD, n=4). The applicability of the proposed method was confirmed to determine MOR in plasma after single administration of MOR (2.5 mg/kg, i.p.) to male Wistar rat. The MOR in plasma could be monitored at 240 min after administration. Pharmacokinetic parameters of MOR obtained from the concentration-time profile were 610±212 ng/ml for Cmax, 62±8 min for T1/2 and 52.9 ±15.5 µg/ml-min for AUCinf.