LIQUID CHROMATOGRAPHIC DETERMINATION OF BUCILLAMINE IN RAT BLOOD BY FLUORESCENT DERIVATIZATION WITH N-(1-PYRENYL)MALEIMIDE AND APPLICATION FOR PHARMACOKINETIC STUDY

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We established a HPLC assay of bucillamine, a disease-modifying anti-rheumatic drug possessing two thiol groups in the structure, by fluorescent derivatization with N-(1-pyrenyl)maleimide (NPM) in rat blood. Also, we investigated the bucillamine disposition kinetics in rats. Rat blood samples were immediately mixed with NPM at room temperature for 15 min and injected into HPLC. Retention time of the bucillamine derivative was 10.2 min. The linearity was displayed for bucillamine concentrations ranging from 2 to 1000 ng/mL ($r^2=0.999$). The lower limit of detection for bucillamine utilizing this method was established at 1.5 ng/mL (signal-to-noise ration of 3:1). The coefficients of variation on the assays were less than 6.7%. The recovery was within 97 to 105%. The values of AUC_{0-8 hr}, MRT_{i.v.}, V_{dss} and CL_{tot} of bucillamine after a single i.v. dose (5 mg/kg) were 1.2 mg·hr/L, 0.73 hr, 3.1 L/kg and 4.2 L/hr/kg, respectively. When bucillamine (10 mg/kg) was p.o. administered to rats, the values of BA, C_{max} and T_{max} were 59%, 0.40 ng/mL and 1.1 hr, respectively. The value of $k_e$ (1.4 hr$^{-1}$) was more than two fold greater compared with that of $k_e$ (0.59 hr$^{-1}$). These results indicate that our HPLC method by fluorescent derivatization of bucillamine with NPM is useful to sensitively, simply and reproducibly determine the bucillamine levels in rat blood and can be applied to pharmacokinetic studies. We strongly expect that the method presented will be clinically utilized.