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Simple and rapid HPLC method for simultaneous determination of atenolol and chlorthalidone in spiked human plasma

Mohamed S. Elgawish, Samia M. Mostafa, Abdalla A. Elshanawane

ABSTRACT

A simple, sensitive and rapid chromatographic method was developed and validated for the simultaneous quantification of atenolol and chlorthalidone in human plasma using hydrochlorothiazide as internal standard (IS). The method utilized proteins precipitation with acetonitril as the only sample preparation involved prior to reverse phase-HPLC. The analytes were chromatographed on Shim-pack cyanopropyl column with isocratic elution with 10mM KH2PO4 (pH 6.0) – methanol (70:30, v/v) at ambient temperature with flow rate of 1mLmin−1 and UV detection at 225nm. The chromatographic run time was less than 10min for the mixture. The calibration curves were linear over the range of 0.1–10μgmL−1. The method was validated in terms of accuracy, precision, absolute recovery, freeze–thaw stability, bench-top stability and re-injection reproducibility. The within- and between-day accuracy and precision were found to be within acceptable limits <15%. The analytes were stable after three freeze–thaw cycles (deviation <15%). The proposed method was specific for the simultaneous determination of atenolol and chlorthalidone in human plasma where there was no interference from endogenous biological substances.