ABSTRACT

An accurate, simple and specific reverse phase HPLC analytical procedure is established and validated for simultaneous determination of caffeine, ephedrine HCl, yohimbine HCl, 17α-methyltestosterone and tocopherol acetate in pharmaceutical formulations. Good chromatographic separations between caffeine, ephedrine HCl, yohimbine HCl, 17α-methyltestosterone and tocopherol acetate and stress induced degradations products were accomplished within 20 minutes using C18 column using water with TEA (triethylamine) pH at 4.2 as mobile phase A and methanol with TEA as mobile phase B, (70-100 % v/v) at variable wavelengths 254nm, 292nm, 210nm on the basis of λ max of caffeine, 17α-methyltestosterone, tocopherol acetate, yohimbine HCl and ephedrine HCl. The flow rate was 1.0 ml/min. Developed method was validated using ICH guidelines. Linearity was from (120 to 720 µg mL-1) for caffeine, (8 to 48 µg mL-1) for ephedrine HCl, (48 to 144 µg mL-1) for yohimbine HCl, (40 to 240 µg mL-1) for 17α-methyltestosterone and (24 to 144 µg mL-1) for tocopherol acetate. The LOD values were found to be 1.16 µg/ml for caffeine, 0.5 µg/ml for yohimbine HCl, 0.8 µg/ml for ephedrine HCl, 0.5 µg/ml for 17α-methyltestosterone and 1.16 µg/ml for tocopherol acetate. The LOQ values were found to be 3.9µg/ml for caffeine, 2.5µg/ml for ephedrine HCl, 1.6µg/ml for yohimbine HCl, 2.6µg/ml for 17α-methyltestosterone and 3.5µg/ml for tocopherol acetate. All the analytes including the degradation products were separated with acceptable peak tailing and resolution. The established method can successfully be used for the routine and simultaneous determination of caffeine, ephedrine HCl, yohimbine HCl, 17α-methyltestosterone and tocopherol acetate in pharmaceutical formulations.