ABSTRACT

Validation of HPLC evaluation of piroxicam in plasma using SPME and precipitation

Rigorous Thesis

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The purpose of this thesis was bioanalytical evaluation of piroxicam using High Performance Liquid Chromatography (HPLC).

Piroxicam was isolated from plasma using SPME and protein precipitation. Plasma was adjusted to pH 2.5. SPME was composed of 20 minutes sorption on PDMS/DVB fiber and 20 minutes desorption into 200 ul of methanol. In the second method the sample of plasma was precipitated with acetonitril, 30 seconds shaken and then 5 minutes centrifuged at 5 000 G. Then the supernatant was removed and analysed with HPLC.

HPLC analysis was on the column with reversed phase C18. The mobile phase consists of water and acetonitrile (60:40 v/v), pH of solution was adjusted to 2.5 using the formic acid. The flow rate was 1 ml/min, the temperature was set at 40°C. The detection was carried out at 333 nm.

SPME and protein precipitation were validated according to FDA. Specificity, accuracy, precision, recovery, linearity, limit of detection a quantification and stability were monitored.