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Determination of MDA by HPLC in Blood of Levofloxacin Treated Rats

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Abstract : Present work demonstrates the applicability of high-performance liquid chromatography (HPLC) with UV-Vis detection for the quantification of malondialdehyde as malondialdehyde-thiobarbituric acid complex (MDA-TBA) in-vivo in rats. The HPLC method for MDA-TBA was achieved by isocratic mode on a reverse-phase C18 column (250mm×4.6mm) at a flow rate of 1.0mLmin−1 followed by detection at 532 nm. The chromatographic conditions were optimized by varying the concentration and pH of water followed by changes in percentage of organic phase optimal mobile phase consisted of mixture of water (0.2% triethylamine pH adjusted to 2.3 by ortho-phosphoric acid) and acetonitrile in ratio (80:20v/v). The retention time of MDA-TBA complex was 3.7 min. The developed method was sensitive as limit of detection and quantification (LOD and LOQ) for MDA-TBA complex were (standard deviation and slope of calibration curve) 110 ng/ml and 363 ng/ml respectively. Calibration studies were done by spiking MDA into rat plasma at concentrations ranging from 500 to 1000 ng/ml. The precision of developed method measured in terms of relative standard deviations for intra-day and inter-day studies was 1.6–5.0% and 1.9–3.6% respectively. The HPLC method was applied for monitoring MDA levels in rats subjected to chronic treatment of levofloxacin (LEV) (5mg/kg/day) for 21 days. Results were compared by findings in control group rats. Mean peak areas of both study groups was subjected for statistical treatment to unpaired student t-test to find p-values. The p value was <0.001 indicating significant results and suggesting increased MDA levels in rats subjected to chronic treatment of LEV of 21 days.

Keywords : malondialdehyde-thiobarbituric acid complex, levofloxacin, HPLC, oxidative stress
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