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Bioanalytical Method Development and Validation of Aminophylline in Rat Plasma Using Reverse Phase High Performance Liquid Chromatography: An Application to Preclinical Pharmacokinetics

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Abstract : Introduction: Aminophylline is a methylxanthine derivative belonging to the class bronchodilator. From the literature survey, reported methods reveals the solid phase extraction and liquid liquid extraction which is highly variable, time consuming, costly and laborious analysis. Present work aims to develop a simple, highly sensitive, precise and accurate high-performance liquid chromatography method for the quantification of Aminophylline in rat plasma samples which can be utilized for preclinical studies. Method: Reverse Phase high-performance liquid chromatography method. Results: Selectivity: Aminophylline and the internal standard were well separated from the co-eluted components and there was no interference from the endogenous material at the retention time of analyte and the internal standard. The LLOQ measurable with acceptable accuracy and precision for the analyte was 0.5 µg/mL. Linearity: The developed and validated method is linear over the range of 0.5-40.0 µg/mL. The coefficient of determination was found to be greater than 0.9967, indicating the linearity of this method. Accuracy and precision: The accuracy and precision values for intra and inter day studies at low, medium and high quality control samples concentrations of aminophylline in the plasma were within the acceptable limits Extraction recovery: The method produced consistent extraction recovery at all 3 QC levels. The mean extraction recovery of aminophylline was 93.57 ± 1.28% while that of internal standard was 90.70 ± 1.30%. Stability: The results show that aminophylline is stable in rat plasma under the studied stability conditions and that it is also stable for about 30 days when stored at -80˚C. Pharmacokinetic studies: The method was successfully applied to the quantitative estimation of aminophylline rat plasma following its oral administration to rats. Discussion: Preclinical studies require a rapid and sensitive method for estimating the drug concentration in the rat plasma. The method described in our article includes a simple protein precipitation extraction technique with ultraviolet detection for quantification. The present method is simple and robust for fast high-throughput sample analysis with less analysis cost for analyzing aminophylline in biological samples. In this proposed method, no interfering peaks were observed at the elution times of aminophylline and the internal standard. The method also had sufficient selectivity, specificity, precision and accuracy over the concentration range of 0.5 - 40.0 µg/mL. An isocratic separation technique was used underlining the simplicity of the presented method.

Keywords : Aminophyllin, preclinical pharmacokinetics, rat plasma, RPHPLC
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