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**Title: Novel SPE-HPLC Methods for Analyses of β-Blockers, Profens and Vitamin B Complex in Human Plasma Using New Generation Columns**

## Abstract

The research work involve the development and validation of fast, reproducible, efficient, economic and environmentally viable chromatographic methods for the analyses of various drugs in human plasma. The drugs included in the research work were, β-adrenergic, non-steroidal anti-inflammatory drugs (NSAIDs) and vitamin B-complex as these are the most commonly prescribed drugs around the world for curing diseases. Although these are considered to be the most reliable medications for these diseases yet these show certain toxic adverse effects and other problems by the gradual accumulation into the body. Therefore, there is a great need for the analyses of these drugs in biological samples (real samples) with low limits of detection. The present thesis describes the development and validation of sample preparation and High Performance Liquid Chromatographic (HPLC) methods for the analyses of these drugs in human plasma. The thesis is divided into six chapters as summarized below.

**Chapter 1:** First chapter describes the importance of the drugs analysis, brief description of chromatography and types of chromatography and sample preparation methodology, automation and miniaturization of sample preparation techniques, advances in the analyses, use of nanoparticles in analysis.

**Chapter 2:** Second chapter presents the details of materials used in the research work. Besides, this chapter also contains the detailed experimental methodologies.

**Chapter 3:** Chapter 3 involves the development and validation of HPLC and solid phase extraction (SPE) method for the separation and identification of mixture of six β-blockers (carazolol, acebutolol, alprenolol, oxprenolol, atenolol and timolol). Optimization of SPE and HPLC conditions was performed to achieve best separation. The recoveries were in the range of 32.3-50.5%, while, LOD and LOQ were 0.5-1.5 μg $mL^{-1}$ and 2.5-8.0 μg $mL^{-1}$, respectively

**Chapter 4:** Chapter 4 deals with the development and validation of dispersive nano solid phase extraction (DNSPE) and HPLC methods for the analyses of mixture of some anti-inflammatory drugs (aspirin, naproxen, ibuprofen, diclofenac, indomethacin and ketoprofen). Iron nanoparticles were synthesized using green method, which was further functionalized with 1-butyl-3-methylimidazolium bromide (ionic liquid) to enhance adsorption efficiency and selectivity. The functionalized iron nanoparticles (sorbent), thus, formed were characterized and further employed in the extraction process. Optimization of NDSPE and HPLC was carried out for optimum separation. The recoveries were in the range 87.4-94.98 %, while, LOD and LOQ were ranged from 0.5-5 μg $mL^{-1}$ and 2.8-45.0 μg $mL^{-1}$, respectively.

**Chapter 5:** Chapter 5 contains the development of solid phase membrane micro-tip extraction (SPMMTE) and HPLC methods for the separation and identification of vitamin B-complex (thiamine, pantothenic acid, riboflavin, pyridoxine, biotin, niacin, folic acid and cynocobalamine). Optimization of SPMMTE and HPLC was performed to achieve the best separation. The recoveries were in the range of 60-83%, while, LOD and LOQ were 1.0-10.0 ng $L^{-1}$ and 10.5-111.0 ng $L^{-1}$, respectively.

**Chapter 6:** Discusses the diagnostic relevance of all three developed methods with their applications in the real world samples.