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Title of the Thesis : **Development of Liquid Chromatographic Methods of Simple and Chiral Drugs Monitoring in Biological Samples**

Abstract

Analysis of drugs in biological sample is an essential requirement for the drugs design and development including the study of pharmacokinetics and pharmacodynamics. Of course, drugs are used for treating various diseases but the presence of their residues in our body for long time is not acceptable as their residues cause some side effects and toxicities. Therefore, the development and validation of new methods for the analyses are always required for providing safe medication to the society. Besides, about 80% drugs are racemic and have been banned in all the developed countries. Therefore, new and effective chiral methods are required for designing and developing homochiral drugs because one of the enantiomers of racemic drugs may be active while the other may be inactive or toxic. Moreover, some enantiomers racemize *in vivo* into their counterparts, which may be toxic or inactive; creating confusion in drug dosages. Due to these facts, it is essential and urgent need of today to develop efficient and effective chiral methods for analyses of racemic drugs in human plasma. Many methods have been used for drugs testing all over the world, which includes chromatography, spectroscopy, crystallization, capillary electrophoresis, membrane, biosensor, biotransformation etc. But the best technique is chromatography due to its high speed, good resolution sensitivity and reproducibility. Three groups of drugs were studied for the analyses in human plasma by SPE-TLC and SPE-HPLC hyphenated technique.

The separation and identification of thin layer chromatography separations of contraceptive drugs (norethindrone acetate and dydrogesterone) have been achieved by using by *n*-hexane-*n*-butanol (90:10, v/v) at 20±1 ºC. The R_f values were 0.82 and 0.73 with 0.40 and 0.10 ngL^{-1} as limits of detection for norethindrone acetate and dydrogesterone, respectively. The R_f values of these drugs on impregnated plates were in the range 0.68-0.94. The best separation was obtained on Cu(II) impregnated plate with compact spots. The percentage recoveries from human plasma by SPE of norethindrone acetate and dydrogesterone were calculated and found to be 79 and 73%, respectively.

The analysis of third generation antibiotics (ceftriaxone, cefixime and cefdinir) in human plasma has been achieved by SPE-HPLC hyphenated technique. The separation of these three antibiotics was achieved on C_{18} column (150 mm × 4.6 mm) of Waters USA by using mobile phase phosphate buffer (50.0 mM, pH5.0)-methanol (80:20, v/v) with a flow rate of 1.0 $mLmin^{-1}$at 20±1°C with detection at 230 nm. The values of capacity factor for all three third generation antibiotics were ranged from 0.35-2.67. The values of separation factor (α) for ceftriaxone-

cefixime, cefixime-cefdinir and ceftriaxone-cefdinir combinations in standard samples were 5.72, 1.34 and 7.65, respectively, while the values of resolution factor (Rs) for ceftriaxone-cefixime, cefixime-cefdinir and ceftriaxone-cefdinir combinations in standard sample were 4.45, 1.34 and 5.77, respectively. The values of detection limits for ceftriaxone, cefixime and cefdinir were 0.1, 0.6 and 0.8 μgL^{-1}, respectively. The percentage recoveries from human plasma by SPE of ceftriaxone, cefixime and cefdinir were 20.92, 25.84 and 37.88%, respectively. The values of separation and resolution for ceftriaxone, cefixime and cefdinir were greater than one indicating a good separation.

Enantiomeric resolution of profens (flurbiprofen and ibuprofen) in human plasma has been achieved by SPE-Chiral HPLC hyphenated technique. The mobile phase used were water-acetonitrile-trifluoroacetic (60:40:0.5, v/v/v) for flurbiprofen and (70:30:0.5, v/v/v) for ibuprofen with a flow rate of 1.5 $mLmin^{-1}$, respectively. The separations were carried out on AmyCoat RP column (150 mm × 46 mm, 3µm particle size) [*tris*-(3, 5- dimethylphenyl) carbamate] of Kromasil, Sweden with 20±1° C temperature and 254 and 236 nm detection for flurbiprofen and ibuprofen, respectively. The optimized condition of chiral separations have been achieved by using different mobile phases and various polysaccharides based CSPs. Chiral-HPLC parameter such as capacity (k), separation (α) and resolution (Rs) factors for the enantiomeric separation of profens (flurbiprofen and ibuprofen) were ranged from 4.57-14.42, 1.10-1.33 and 1.01-1.49, respectively. The values of separation and resolution factors were greater than 1.0 indicating the complete resolution; with sharp peaks of enantiomers of both profens. The limits of detection of both profens were calculated and ranged from 1.0 to 2.5 μgL^{-1}. The percentage recoveries of both enantiomers of these drugs from human plasma were 60.80, 55.60 % for (R)- and (S)-flurbiprofen and 35.60, 30.0% for (R)- and (S)-ibuprofen, respectively.

The limits of detection were quite good and no extra spot and peak appear in the TLC, Simple HPLC and Chiral-HPLC chromatograms, showing the selective nature of SPE method. The work describing the analyses of above cited drugs in human plasma is useful in pharmacokinetic and dynamic studies for the development of safe medication to our society.

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