Name of Scholar : Afzal Hussain

Name of Supervisor : Dr. Imran Ali

Name of Co-supervisor : Prof. Kishwar Saleem

Department : Chemistry

Title of the Thesis : **Evaluation of Chromatographic Methods for Analyses of Simple and Chiral Drugs in Human Plasma**

Abstracts

New methods development and validation for drugs and pharmaceutical analysis are integral part in pharmaceutical industries and biological sciences. 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 mild toxicities, which may be lethal for the human being. Besides, quality control in pharmaceutical industries is demanding with development of novel methods of analysis for various drugs and pharmaceuticals. Therefore, the development and validation of new methods are always required for providing safe medication to the society. Besides, quality control requires monitoring in each laboratory with inter-calibration among laboratories as an extremely useful exercise. *In vitro* profiles obtained from dissolution rate studies have also been used in the successful characterization of *in vivo* behaviour of drugs. Besides, about 80% drugs are racemic and have been banned in all developed countries. Therefore, new and effective chiral methods are required for designing and developing homochiral drugs as one of the enantiomers of racemic drugs may be active while the other may be inactive or toxic. Basically, enantiomers bind to human body receptors in stereo-specific fashion resulting into different pharmaceutical potencies. Moreover, some enantiomers racemize *in vivo* giving to other antipodes, which may be toxic or inactive creating confusion in drug dosages. Due to these facts, it is essential and urgent need to develop efficient and effective chiral methods for analyses o f 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.

Development and validation of thin layer chromatography (TLC) and solid phase extraction (SPE) for the separation and identification of simple mixtures of anti-depressant drugs (fluvoxamine maleate, paroxetine and sertraline) was achieved by using 2-propanol-DCM (70:30, v/v) at 30±1 ºC. The R_f values were 0.44, 0.22 and 0.68 for fluvoxamine maleate, paroxetine and sertraline, respectively. The metal ions used were Fe (II), Ni(II), Cu(II) and Zn(II) of 0.1% concentration, separately. The R_f values of these drugs on impregnated plates were in the range 0.18 to 0.64. The separation of these drugs on plain and impregnated plates was compared and it was found that the best separation was on Ni(II) impregnated plate with compact spots. The limits of detection of these drugs were also calculated and found to be 0.1-0.2 $ngmL^{-1}$ for both plain and impregnated plates. The values of the resolution data (R) for

fluvoxamine maleate, paroxetine and sertraline were greater than 1.0 and, hence, the complete separation. The solid phase extraction conditions of these dugs were phosphate buffer (50.0 mM, pH 9.0) with 0.10 $mLmin^{-1}$ as flow rate. As a result of an extensive experimentation the best eluting solvent was methanol containing 0.1% acetic for all anti-depressant; with 0.1 $mLmin^{-1}$ flow rate. The percentage recoveries of fluvoxamine maleate, paroxetine and sertraline drugs were calculated and found to be 41%, 35% and 33%, respectively.

The separation and identification of anti-inflammatory drugs (flurbiprofen, ibuprofen and ketoprofen) in human plasma have been achieved of a mixture of these drugs by using high performance liquid chromatographic (HPLC) technique. Attempts have been made to achieve the best chromatographic separation with the help of water : acetonitrile (55:45, v/v) and the pH of mobile phase was maintained 3.0 by trifluoro acetic acid and the flow rate was 0.5 $mLmin^{-1}$ at 20±1°C with detection at 225 nm. The best results have been achieved on the C_{18} column (150 mm×4.6 mm) of Waters USA. The values of capacity factor for all three anti-inflammatory drugs in standard and plasma samples were ranged from 0.69-1.99 and 0.47-1.50 respectively. The values of selectivity factor (α) for Ketoprofen-Flurbiprofen, Flurbiprofen-Ibuprofen and Ibuprofen-Ketoprofen combinations in standard samples were 2.04, 1.41 and 2.88, respectively while these values in human plasma samples were 1.99, 1.00 and 2.10, respectively. The values of resolution factor (Rs) for Ketoprofen-Flurbiprofen, Flurbiprofen-Ibuprofen and Ibuprofen-Ketoprofen in standard sample were 3.06, 1.77 and 4.25, respectively and in human plasma sample were 3.00, 1.50 and 4.10, respectively. The solid phase extraction conditions of these drugs were phosphate buffer (50.0 mM, pH 6.0) with 0.10 $mLmin^{-1}$ as flow rate. As a result of an extensive experimentation the best eluting solvent was methanol for all anti-inflammatory; with 0.1 $mLmin^{-1}$ flow rate. The recovered concentrations of ketoprofen, flurbiprofen and ibuprofen from human plasma were 70, 65 and 76% respectively. The values of selectivity and resolution factor for ketoprofen, flurbiprofen and ibuprofen were greater than one indicating a good separation.

Development and validation of chiral HPLC methods for enantiomeric resolution of β-adrenergic blockers (alprenolol, carazolol, metoprolol, oxprenolol and propranolol) in human plasma. The column used for the study was Cellucoat [(*tris*-(3,5-dimethylphenyl carbamate)] column of Kromasil. The mobile phase used was *n*-heptane-ethanol-diethylamine (90:10:0.2, v/v/v). The mobile phases used in this study were different combinations of *n*-heptane-ethanol-diethylamine (90:10:0.2, v/v/v), which were used at 1.0 $mLmin^{-1}$ and 2 $mLmin^{-1}$ flow rates at 27±1 °C temperature with detection at 225 nm. Chiral-HPLC parameter such as capacity (*k*), separation (α) and resolution (*Rs*) factors for the enantiomeric resolution of β-adrenergic blockers (alprenolol, carazolol, metoprolol, oxprenolol, propranolol) were ranged from 0.46-6.25, 1.12-2.19 and 1.06-9.50, respectively. The values of separation and resolution factors were greater than 1.0 indicating the complete resolution; with sharp peaks of all β-adrenergic blockers. The limit of detection of all β-adrenergic blockers were calculated and ranged from 1.0-2.5 μg/L. All β-adrenergic blockers were separated using 1.0 $mLmin^{-1}$ flow rate except carazolol, which was resolved by using 2.0 $mLmin^{-1}$ flow rates.

The solid phase extraction conditions of these β-adrenergic blockers were ammonia buffer (50.0 mM, pH 9.0) with 0.10 $mLmin^{-1}$ as flow rate. As a result of an extensive experimentation the best eluting solvent was methanol containing 0.1% acetic acid for all β-adrenergic blockers; with 0.1 $mLmin^{-1}$ flow rate. The binding concentration differences of S-(-) and R-(+) enantiomers of propranolol, oxprenolol, carazolol, alprenolol and metoprolol were 3.0

0.12, 0.08, 0.05 and 0.01, respectively. Therefore, it may be assumed that the order of their activities may be propranolol >oxprenolol >carazolol > alprenolol> metoprolol.

The results presented in this thesis indicate that the reported SPE-TLC and SPE-HPLC methods are selective, efficient, reproducible, inexpensive, robust, having low limits of detection and quantification, which may be applied to any biological samples for monitoring of above cited drugs. The results of TLC are of prime importance as it can be easily used in developing and under developed countries for the welfare of human beings. The values of separation and resolution factors were greater than 1.0 indicating a good resolution. The different binding concentrations of the above cited drugs with human plasma protein confirmed their pharmaceutical activities. More binding of S-(-)-enantiomers of β-adernergic blockers with plasma proteins indicates their higher pharmaceutical activities in comparison to their R-(+)-antipodes. The limits of detection were quite good and no extra spot and peak appeared in TLC, Simple HPLC and Chiral-HPLC chromatograms, showing the selective nature of SPE methods. The present work describing the analyses of above cited drugs in human plasma is also useful for pharmacokinetic and pharmacodynamic for designing more effective drugs with least side effect.

Email I.D.: afzalchem@gmail.com