SYNOPSIS OF THESIS ENTITLED
DEVELOPMENT AND VALIDATION OF NEWER RP-HPLC METHODS
FOR THE ESTIMATION OF SELECTED DRUGS

Synopsis Submitted to
ACHARYA NAGARJUNA UNIVERSITY

In partial fulfillment for the award of the degree of

DOCTOR OF PHILOSOPHY
IN
CHEMISTRY

By
Mr. A.V.D.NAGENDRA KUMAR  M.Sc., M.Phil.,

Under the Supervision of
Prof. M. V. BASAWESWARA RAO
M.Sc., M.Phil.,Ph.D, MNASC.,MRSC

Department of Chemistry,
Krishna University, Machilipatnam, Andhra Pradesh

DEPARTMENT OF CHEMISTRY
ACHARYA NAGARJUNA UNIVERSITY
NAGARJUNA NAGAR
GUNTUR, A.P., INDIA
AUGUST - 2011
SYNOPSIS

DEVELOPMENT AND VALIDATION OF NEWER RP-HPLC METHODS FOR THE ESTIMATION OF SELECTED DRUGS

In industry, the quality assurance and quality control departments play major role in bringing out a safe and effective drug or dosage form. The pharmaceutical industry is to deliver market safe, efficacious product that fulfill medical needs of public.

Development of new analytical methods for the determination of drugs quantitatively and qualitatively in pharmaceutical dosage forms is more important in pharmacokinetic, toxicological and biological studies. The current good manufacturing practices (CGMP) and the Food Drug Administration (FDA) guidelines insist for adoption of sound methods of analysis with greater sensitivity and reproducibility. The number of drugs invented and implemented is constantly increasing to cater the needs of public. This requires new methods for controlling their quality. Most of the modern drugs are potential and hence sensitive methods are needed for their estimation in pharmaceutical formulations or in bulk drugs.

The present investigation deals with the development of new analytical methods for the quantitative determination of selected drugs in bulk and its Pharmaceutical Dosage forms.

Chapter - 1 embodies brief information on general analytical methodology, selection of drugs and also gives information on systemic procedure to be followed for development of new chromatographic methods for the various drugs selected by author for the study are Zopiclone, Pizotifen, Sirolimus, Stanozolal, Stavudine, Buspirone, Quietiapine, Telmisartan, Darunavir, Diacerin, Carvidiol and livetaracetam. The later part of the chapter contains survey of literature about the analytical methods.

Chapter – 2 describes to optimize the RP-HPLC parameters, several mobile phase compositions were tried for the determination of Zopiclone. A satisfactory separation and good peak symmetry was found in a mixture of Acetonitril: Methanol: THF: 0.1%OPA in the ratio of 45:20:5:30 v/v and 1.0 mL/min flow rate proved to be
better than the other mixtures in terms of resolution and peak shape. The optimum wavelength was set at 303 nm. The number of theoretical plates was found to be 5557.8, which indicates efficient performance of the column. The calibration curve was obtained for a series of concentration in the range of 0.2-1.4µg/ml and it was found to be linear.

The system suitability parameter like capacity factor, asymmetry factor, tailing factor and number of theoretical plates were also calculated. It was observed that all the values are within the limits.

Chapter – 3 deals with the study of Pizotifen. A mixture of orthophosphoric acid, acetonitrile and methanol was selected as mobile phase and the effect of composition of mobile phase on the retention time of Pizotifen was thoroughly investigated. A system suitability test was applied to representative chromatograms for various parameters. Ten points graphs was constructed covering a concentration range 5-30 ppm. The standard deviation of the slope and intercept were low. The calibration curve was obtained for a series of concentration in the range of 0.5-30 ppm and it was found to be linear.

Chapter – 4 narrates the efficiency of a chromatographic separation for the determination of Sirolimus the quality of the chromatography was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor > 2.0, tailing factor ≤ 2.0 and theoretical plates > 2000. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%.

Standard curves were constructed using six standard concentrations in a range of 5, 10, 15, 20, 25, 30 µg/ml for Sirolimus. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The proposed method for the assay of Sirolimus in tablets or capsules is very simple and rapid. It should be emphasized that it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness.
Chapter – 5 incorporates to develop a precise, accurate and suitable RP- HPLC method for the simultaneous estimation of Stanozolol. Different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination. Mixture of Methanol and Water (90:10 v/v) was selected as mobile phase. A system suitability test was applied to representative chromatograms for various parameters. The results obtained were within acceptable limits.

Chapter – 6 includes the work to develop a precise, accurate and suitable RP- HPLC method for the simultaneous estimation of Stavudine. Different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate. The Ultra violet spectra of Stavidine showed that the drug absorbs appreciably at 267nm was selected as the detection wave length in liquid chromatography. Satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase Methanol: Acetonitrile: 0.1 %OPA (40:50:10, v/v/v).

The retention time of Stavudine was found to be 6.8 min, which indicates a good base line. The calibration curve for Stavudine was obtained over the range of 0.5-3.0 ppm, and it was found to be linear with r²=0.999. The results obtained were within acceptable limits.

Chapter – 7 Deals with the precise, accurate and suitable RP- HPLC method for the simultaneous estimation of Buspirone. Different mobile phases were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination. The system suitability method acceptance criteria set in each validation run were, capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2000 13. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%.

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise. To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100% and 150%.
Chapter – 8 deals with the precise, accurate and suitable RP- HPLC method for the simultaneous estimation of Quetiapine. System suitability tests were carried out as per USP XXIV. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. Standard curves were constructed using five standard concentrations in a range of 2, 4, 6, 8, 10 ppm for Quetiapine. The linear regression equation was \( y = -1299 + 89210x \) \((r= 0.999)\). The R.S.D. values of the slope were 20448.02 \((n=3)\) and the R.S.D. of \(y\)-intercept was -991.76 \((n=3)\).

The proposed method for the assay of Quetiapine in tablets or capsules is very simple and rapid. It should be emphasized that it is isocratic and the mobile phase do not contain any buffer.

Chapter – 9 narrates with the satisfactory separation and good peak symmetry for Telmisartan was found in a mixture of Acetonitril: Methanol: Acetonitrile : 0.1%OPA in the ratio of 80:15:05 v/v and 1.5 mL/min flow rate proved to be better than the other mixtures in terms of resolution and peak shape. The optimum wavelength for detection was set at 256nm. The retention times were 2.7 min for Telmisartan. The number of theoretical plates was found to be 6638.20. A system suitability test was applied to representative chromatograms for various parameters.

The calibration curve was obtained for a series of concentration in the range of 2-12µg/ml and it was found to be linear. Seven points graphs was constructed covering a concentration range 2-12µg/ml. The standard deviation of the slope and intercept were low. Calibration curve found to be linear with \( r^2=0.999, \) Intercept \((-150323.5)\) and Slope\( (45459.12)\) respectively. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. The proposed method has been applied to the assay of commercial tablets containing Telmisartan. The results \((18.57\%)\) presented good agreement with the labeled content.

Chapter – 10 deals with the efficiency of a chromatographic separation for Darunavir was monitored by applying the system suitability tests. It was assessed by replicate analysis of two injections of the drug at a concentration of 30ppm. The acceptance criterion was ± 2% for the percent coefficient of variation (%CV) for the peak area and retention times for Darunavir. The number of theoretical plates should not be
less than 2500 and the tailing factor should not be more than 2.0. The peak purity of Darunavir was assessed by comparing the retention time (Rt) of standard and the sample. Linearity ranges for Darunavir found to be 10-50ppm.

Known amounts of standard Darunavir added to pre-analyzed samples and were subjected to the proposed HPLC method at 50%, 100% and 150% to evaluate the degree of accuracy. Results of recovery studies are shown range 99.00-101.45%.

Chapter – 11 deals with the study of Diacerein and it is non-polar. Non-polar compounds preferably analyzed by reverse phase columns. Among C8 and C18, C18 column was selected. So the elution of the compound from the column was influenced by polar mobile phase. Mixture of water and methanol was selected as mobile phase. Retention time of Diacerein was thoroughly investigated.

The sample solution was prepared and the percentage relative standard deviation (%RSD) was found for Intraday-0.916, Interday-0.399) to be less than 2% for within a day and day to day variations. To check the degree of accuracy of the method, recovery studies were performed. Known amounts of standard were added to pre-analyzed samples and were subjected to the proposed HPLC method. Results of recovery studies are shown range 99.00-101.45%.

The proposed method has been applied to the assay of commercial tablets (DYCERIN - 50 mg) containing Diacerien. After analysis test result assay of Diacerien in Tablet is 15.7% and is very close to the labeled amount. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The RSD for all parameters was found to be < 2 indicates the validity of method and assay results obtained by this method are in fair agreement.

Chapter – 12 deals with the study of Carvedilol. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor =2.0 and theoretical plates >2000. In all cases, the relative standard deviation (R.S.D) for the analyte peak area for two consecutive injections was < 2.0%.

The calibration curve for Carvedilol was obtained over the range of 50-100ppm, and it was found to be linear with r = 0.999. The intraday and inter day precision study of Carvedilol was carried out by estimating the corresponding responses 3 times on the
same day and on 3 different days (freshly prepared) for 3 different concentrations of Carvedilol (60, 80, 100 ppm). The standard deviation (Intraday-0.179, Interday-0.177), and coefficient of variation was calculated and they are within the acceptance limit. Results of recovery studies are shown range 99.00-101.45%.

Chapter – 13 deals with the study of Levetiracetam. A satisfactory separation and good peak symmetry was found in a mixture of Methanol: Acetonitrile in the ratio of 80:20 v/v. The optimum wavelength for detection was set at 208 nm at which much better detector responses for drug was obtained. The retention times were 2.58 min for Levetiracetam. Standard curves were constructed using six standard concentrations. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The validated method was applied for the assay of commercial tablets (KEPPRA-250 mg) containing Levetiracetam. Sample was analyzed for five times after extracting the drug as mentioned in assay sample preparation of the experimental section. The results presented good agreement with the labeled content. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. It could be used for the rapid and reliable determination of Levetiracetam in tablet formulation.

Hence this thesis is framed with an Introduction followed by Drug profile, Literature Survey, Experimental, RP-HPLC Method development, Validation of the proposed method, Discussion on the Results and appropriate references are reported. The procedures offered by the author are relatively simple and revealed good linearity, accuracy and reproducibility.