Analytical Techniques for the estimation of Pharmaceutical Drugs in Pure and tablet dosage forms

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**SYNOPSIS OF THE Ph.D.THESES**

Drug is a pharmacological substance which when absorbed into a living organism may modify one or more of its functions and is intended for medicinal usage to assist the diagnosis, cure, treatment, or prevention of disease or ailment. In general the term drug refers to a substance taken for a therapeutic purpose. The Drugs are used in various dosage forms in therapy. They are formulated as tablets, binders, adhesives, capsules, oily, aqueous suspensions, ointments and creams. Every country has legislation on bulk drugs and their pharmaceutical formulations that set standards and obligatory quality indices for them.

These regulations are presented in separate articles - general and specific - relating to individual drugs and are published in the form of a book called pharmacopoeia (e.g. Indian Pharmacopoeia, United Kingdom and United States Pharmacopoeia, European Pharmacopoeia, Martindale extra pharmacopoeia, Merck Index, Japan Pharmacopeia.

It involves the introduction of more refined and sensitive methods of physiochemical analysis [18-20] such as colorimetry, spectrophotometry covering UV, visible and IR regions, fluorimetry or turbidimetry, NMR and Mass, and chromatography that enables one to assay the quality of drugs more accurately and with the smallest consumption of the analyte, reagent and time.

The modern methods of choice (HPLC, GLC, NMR and Mass) for purity assay involve sophisticated equipment which are costly and pose problems of maintenance. Hence they are not in the reach of most laboratories and small scale industries. The visible spectrophotometric or colorometric, or fluorimetric methods are very simple, cheap and are easy to carry out. The limitations in the visible spectrophotometric methods of analysis are dependant up on the chemical reactions rather than the sophistication of the instruments. The visible spectrophotometry is selected as a tool for the assay of selected drugs in the present investigation.

The nature of organic drugs towards reactivity depends on the presence of functional groups in their molecules. Knowing the reactions of functional groups, one can
easily analyze any drug with a complicated structure. The structural features (official names, chemical names, structures and analytically useful functional groups) of the selected drugs in the present investigations are given in corresponding chapters. Literature survey on those drugs showed that there are very few visible spectrophotometric methods of analysis at the time of commencement of these investigations.

The analytically useful functional groups of selected drugs have not been fully exploited for developing suitable visible spectrophotometric methods for their assay. The chemical features of selected drug molecules still offer a lot of scope for the development of new visible spectrophotometric methods hopefully with better sensitivity, selectivity, precision and accuracy. The author has made some attempts in this direction and succeeded in developing some new methods.

In CHAPTER-II, Nevirapine was discovered by Hargrave et al. at BoehringerIngelheim Pharmaceuticals, Inc., one of the Boehringer Ingelheim group of companies. It is covered by corresponding foreign patents. Nevirapine was the first NNRTI approved by the U.S. Food and Drug Administration (FDA). At present nevirapine is an official drug in Indian Pharmacopoeia British Pharmacopoeia and United States Pharmacopoeia. Nevirapine is an anti-HIV of the non-nucleoside reverse transcriptase inhibitor (NNRTI). It is more potent than zidovudine on HIV-1, but do not inhibit HIV-2. Viral resistance to these drugs develops by point mutation and cross resistance is common. The linear response was obtained with acceptable correlation co-efficient. No significant interference seen in specificity study.

This method was fully validation of nevirapine and related substances with the precision, specificity, accuracy, linearity, ruggedness, robustness and solution stability. The values obtained for nevirapine, related compound A, B and impurity C was within the acceptance criteria. Hence the findings indicate that the developed method is precise, specific, accurate, linear, robust and rugged. This method can be used for the routine analysis of nevirapine and its related substances in tablet dosage form.

In CHAPTER-III Cyproheptadine hydrochloride (CYP), chemically known as 4-(5Hdibenzo [a,d]-cyclohepten-5-ylidene)-1-methylpiperidine hydrochloride, is a sedating
antihistamine ithanthimuscarinic, serotonin-antagonist, and calcium-channel blocking action in pancreatic islet cells and smooth muscle It is used to treat some hormonal disorders and may also be used for treating side effects of taking antidepressants.CyproheptadineHCl is a white to off white powder.

It is sparingly soluble in water and slightly soluble in methanol and ethanol. The drug is official in Indian Pharmacopeia which describes anSpectrophotometric method for its assay in tablet. The United Sates Pharmacopeia describes on-aqueous titration with perchloric acid as titrant where the end point is located visually using crystal violet as indicator. Literature survey revealed the availability of few methods for the assay of Cyproheptadine in pharmaceutical formulations. Liquid chromatography- mass spectrometry (LCMS) gas liquid chromatography and high performance liquid chromatography (HPLC) have been used to assay Cyproheptadine.

Recently, HPLC has been used for the assay of Cyproheptadine in feedstuff. Application of visible spectrophotometric methods, derivative UVspectrophotometry for the assay of Cyproheptadine in two- component system [24] has also been reported. Ion-selective based potentiometer is another technique which has found application in the analysis of CPH-containing tablets.

The drug has been assayed by potentiometry using Cyproheptadinetetraphenylborate Cyproheptadine-dinonylnaphthalenesulphonicacid, and Cyproheptadine-tetrakis (4-chlorophenyl) borate as electro active compounds. We have developed a fast, simple and reliable analytical method for determination of CyproheptadineHCl in pharmaceutical preparation using RP-HPLC. There is no interference of blank at the retention time of CyproheptadineHCl. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with a good accuracy and precision. It allows reliably the analysis of CyproheptadineHCl in bulk, its pharmaceutical dosage forms.

In CHAPTER-IV Loperamide is a synthetic piperidine derivative, is an opioid drug effective against diarrhea resulting from gastroenteritis or inflammatory bowel
disease. In most countries it is available generically and under brand names such as Lopex, Imodium, Dimor, Fortasec and Pepto Diarrhea Control.

It was developed at JanssenPharmaceutical. Loperamide is an opioid-receptor agonist and acts on the µ-opioid receptors in the myenteric plexus of the large intestine; by itself it does not affect the central nervous system like other opioids. It works by decreasing the activity of the myenteric plexus, Loperamide also decreases colonic mass movements and suppresses the gastrocolic reflex. Loperamide molecules do not cross the blood-brain barrier in significant amounts, and, thus, it has no analgesic or euphoric properties. Any that do cross the blood-brain barrier are quickly exported from the brain by P-glycoprotein also known as multidrug resistance protein.

Tolerance in response to long-term use has not been reported. However, loperamide has been shown to cause a mild physical dependence during preclinical studies, specifically in mice, rats, and rhesus. Symptoms of mild opiate withdrawal have been observed following abrupt discontinuation of long-term therapy with loperamide. Comprehensive literature survey reveals that several analytical methods have been reported for the estimation of LPD which includes Reverse Phase HPLC Spectrophotometry LC-MS spectrofluorimetric, electro kinetic chromatograph.

This proposed method was validated with respect to selectivity, linearity, precision, and accuracy, limit of quantitation (LOQ) and limit of detection (LOD) according to ICH requirements to show it could be used for determination of LPD in pharmaceutical formulations.

Result and Conclusion in this study of the system precision theoretical plates 8291 for loperamide hydrochloride peak was obtained, asymmetry for loperamide hydrochloride peak 1.20 was obtained, the % RSD of area 0.20 for loperamide hydrochloride peak was obtained and % RSD of RT’s 0.12 for loperamide hydrochloride peak was obtained.

In the study of the robustness (variation in mobile phase) theoretical plates for loperamide hydrochloride peak 9618 was obtained, asymmetry for loperamide hydrochloride peak 1.17 was obtained, % RSD of area for loperamide hydrochloride peak
0.13 was obtained, % difference calculated for assay content from two preparations of test preparation 0.02 was obtained. The Cumulative % RSD of the assay contents obtained from Method precision and robustness study Mobile phase variation (+) 0.08 was obtained.

In CHAPTER-V, Loratadine is a derivative of azatadine and a second-generation histamine H1 receptor antagonist used in the treatment of allergic rhinitis and urticaria. Unlike most classical antihistamines (histamine H1 antagonists) it lacks central nervous system depressing effects such as drowsiness. IUPAC Name ethyl 4-{13-chloro-4-azatricyclo9.4.0.0pentadeca-1, 3, 5, 7, 12, 14-hexaen-2-ylidene} piperidine-1carboxylate. Its molecular formula is C_{22}H_{23}ClN_{2}O_{2} and its molecular weight is 382.883.

The chemical structure is the Analysis of Loratadine [LRD] in bulk drug and in tablet dosage forms a rapid and sensitive Reverse Phase High Performance Liquid Chromatographic [RP-HPLC] method was developed for the estimation of Loratadine [LRD] in pure and its capsule dosage forms. The method was validated as per International Conference on Harmonization [ICH] guidelines.

The mobile phase used in this study is a mixture of (0.01M) Dibasic potassium phosphate, methanol and acetonitrile (7:6:6)% v/v. Stationary phase was Chemsil C8 reverse phase column (150×4.6mm, 5μm) dimensions at 30ºc temperature. The analysis was performed with run time of 30.0 minutes at a flow rate of 1.00ml/min. The [LRD] was monitored at 254nm with UV detection and [LRD] was eluted at 22.20min.

The method was linear (r^2 =0.999) at concentration ranging from 25 to 150μg/ml, precise (intra-day relative standard deviation [RSD] and inter-day RSD values < 1.0%), accurate (mean recovery = 99.5%), estimated from linearity by regression respectively. The results showed that the proposed method is suitable for the precise, accurate and rapid determination of [LRD] in bulk, its capsule dosage forms.

The objectives of this study were, therefore, to develop a simple, accurate, sensitive and validated RP-HPLC method for the quantification loratadine in bulk drug
and in tablet dosage forms with good sensitivity. Method validation for the developed method was done according to ICH guidelines.

The composition and flow rate of the mobile phase was programmed from mother pump and the mobile phase for the system suitability, buffer, acetonitrile and methanol (7,6,6.) was passed through the 0.45µm membrane filter using millipore HPLC solvent filtration assembly, was delivered at 1.0mL/min for column stabilization. During this period, the base line was continuously monitored.

The wavelength of detection was selected at 254nm. The prepared dilutions containing concentrations of Loratadine in the range 25.0 -150µg/mL were injected into the chromatograph. The stability of the solution of Loratadine during analysis was determined by repeated analysis of samples during the course of the experiment of the same day and also on different days after storing at laboratory bench conditions and in the refrigeration.

Chromatogram parameters, retention time and asymmetry factor were standardized. A chromatogram indicating the separation of Loratadine is given. The retention time for Loratadine is 22.20min. The amount of the drug present in each pharmaceutical formulation was calculated through peak area ratio of component by making use of the standard calibration curve. The method was validated following the parameters such as specificity, linearity, precision, and accuracy, limits of detection and quantitation and robustness, following the ICH guidelines (ICH).

The specificity of the method was evaluated with regards to interference due to the presence of excipients in the pharmaceutical formulation. The placebo samples consisted of all the excipients without the active substance. Then, the specificity of the method was established by determining the peak purity of Loratadine in samples using a UV detector, ranging between 190-400nm.

The determined specificity with respect to sample compounds the responses of standard and sample solution was compared. No interferences were detected at the retention times of Loratadine in sample solution.

The precision of the proposed method was investigated by intra-day and inter-day determinations of Loratadine at three different concentrations of Loratadine (4, 8 and 12
μg/mL). The intra-day studies were performed in one day (for each level n=5) and inter-day studies in five days over a period of two weeks. The intra and inter-day precisions expressed as relative standard deviation values (RSD %) for Loratadine were found to be within 0.87-1.99 % and 0.99-2.03 %, respectively. The data proved good precision for the developed method.

Accuracy is further assessed by recovery experiments. Recovery experiments were performed by applying the standard-addition technique. The recovery was assessed by determining the agreement between the measured standard concentration and added known concentration to the sample. The test was done by spiking the pre-analyzed tablet powder with pure Loratadine at three different levels [50, 100 and 150 % of the content present in the tablet powder (taken)] and the total was found by the proposed method.

Each test was repeated three times. In all the cases, the recovery percentage values ranged between 99.53 and 99.89. Closeness of the results to 100% showed the fairly good accuracy of the method. The results are shown in Table 1. The reported RP-HPLC method developed by the author for the analysis of Loratadine [LRD] was proved to be simple, rapid and reproducible. The validation data indicate good precision, accuracy and reliability of the developed RP-HPLC method.

The developed method offers several advantages in terms of simplicity in mobile phase, mode of elution, easy sample preparation steps and comparative short run time which makes the method specific and reliable for its intended use in routine analysis determination of Loratadine [LRD] in tablet dosage forms.

The references are indicated serially at appropriate places in the body of thesis and also given at the end of each chapter.
LIST OF PUBLICATIONS

A) PAPERS IN JOURNALS

1. T.Sujatha¹, K.Balmuralikrishna² and R.Ramesh Raju*
   “Visible Spectrophotometric determination of Loratadine through oxidative coupling reaction in bulk and its pharmaceutical preparations”
   International Journal of Chem Tech Research Vol.6, No.2, pp 1091-1096,

2. T.Sujatha¹, K.Balmuralikrishna² and R.Ramesh Raju*
   “A Validated RP-HPLC Method for the estimation of Loperamide Hydrochloride in Tablet dosage forms”
   International Journal of ChemTech Research Vol.6, No.2, pp 1097-1102,

3. T.Sujatha¹, K.Balmuralikrishna² and R.Ramesh Raju*
   “RP-HPLC method for the estimation of loratadine [LRD] in bulk drug and in tablet dosage forms”

B) PAPERS IN CONFERENCES/SEMINARS

1. T.Sujatha¹, K.Balmuralikrishna² and R.Ramesh Raju*
   “Visible Spectrophotometric determination of Loratadine through oxidative coupling reaction in bulk and its pharmaceutical preparations”
   International Journal of Chem Tech Research Vol.6, No.2, pp 1091-1096,