Chapter VI

Summary
&
Conclusion
6. SUMMARY AND FINDINGS

The analysis of impurities in drug substances, beginning with the initial screening and ending with the use of validated methods in routine quality control and quality assurance, is becoming an increasingly challenging task along the pharmaceutical value delivery chain. This compendium offers guidance on how Rapid Resolution Liquid Chromatography (RRLC), in combination with mass spectrometry, can improve overall workflow for analyzing and identifying impurities in drug substances. The validation processes development of new method validation in pharmaceutical analysis is one of the important aspects. A method of analysis is characterised by its performance parameters, which have to be assessed if they are to provide the correct performance values. These performance values must be in accordance with previously defined requirements the method of analysis should satisfy. But above all, the performance parameters primarily depend on the type of method and its intrinsic characteristics. Quantitative methods of analysis provide information not only about the composition but also about the concentration of the analyst present in the sample and they often require more complex analytical techniques to obtain more accurate and reliable information about the sample.

Among all separation tools, high performance liquid chromatography (HPLC) takes a central role in pharmaceutical analysis. Liquid chromatography (LC) is a separation method of the great importance to the chemical, pharmaceutical and biotechnological industry. The usefulness and popularity of HPLC was increased by the possibility to automate and computerize the systems providing unattended operations and high sample capacities. In analytical chromatography the aim of the separation is to obtain quantitative and qualitative information about the compounds of interest (analyst) in a sample. Analytical chemists have to analyze a variety of complex samples often originating in difficult matrices to answer questions about the quality and quantity of different analyst. A complex sample often contains a wide range of components with varying solubilities. Therefore the sample preparation and the separation methods must be highly selective and sensitive. These requirements are satisfied with
HPLC especially if combined with an advanced detection technique such as diode array detection (DAD) or mass spectrometry (MS).

The HPLC method developed may have some intrinsic problems which cannot be taken in pharmaceutical industry as the products assessed by faulty to the market and were administered to already ill patients. And also the methods need to be transferred to different laboratories once they are developed, where a less skilled analyst may be executing the methods to analyse the samples. Hence the methods need to be tested and proven for their accuracy, precision, rugged and robustness before they are implemented or transferred to other laboratories. The whole validation process is done by an expert supervisor. Thus, “validation is the process by which it is established by laboratory studies, that the performance characteristics of the method meet the requirements for the intended application”.

Ultra performance liquid chromatography (UPLC) is a new category of analytical separation science that retains the practicality and principles of HPLC, while increasing the overall interlaced attributes of speed, sensitivity and resolution. During the past 30 years or so, high performance liquid chromatography (HPLC) has proven to be the predominant technology used in laboratories worldwide. One of the primary drivers for the growth of this technique has been the evolution of the packing materials used to effect the separation. Increasing demand for greater pharmaceutical analysis throughput prompted the testing of the waters ACQUITY Ultra Performance LC (UPLC™). This system claims to provide faster analyses through the use of a novel separation material of very fine particle size (1.7 μm) and unique core chemistry. To effect fast separations on this material, the column hardware and instrument have significant design modifications from typical HPLC. The UPLC operates at higher pressures (up to 15,000 psi.), injects samples into a smaller system dwell volume, and captures detector signals at high data rates for fast eluting peaks. A new needle design has been claimed to substantially reduce carryover which can aid in the lowering of limits of quantisation (LOQ).

The pharmaceutical industry routinely uses high performance liquid chromatography (HPLC) to quantititative levels of impurities at the mandated level of 0.1%. The pharmaceutical drug substances and their impurities are often
structurally similar and therefore may co-elute. The evaluation of peak purity is a key component of method development and validation. Analytical techniques typically used to increase the analyst's confidence in the purity of chromatographic peaks include orthogonal separations, selectivity testing using potential impurities and fraction collections followed by alternate testing. These off-line techniques can be time consuming and ineffective, especially at the early stages of drug development when impurities are unknown. Diode array detection (DAD) has also been widely used to provide chromatographic peak purity determinations. Although it was demonstrated that DAD can provide rapid on-line determinations, disadvantages such as inability to consistently detect an impurity below 0.5 per cent co-eluting with a high level main analyte and a requirement for some separation of analytes were acknowledged. Coupling HPLC with mass spectrometry (LC-MS) offers an alternative mode of detection which might be exploited for rapid on-line HPLC peak purity assessment. Unlike DAD, LC-MS has the potential, with the exception of isobaric impurities, to provide detection capabilities for all impurities. Mass spectrometric detection offers the added ability to provide mass and structural information on the co-eluting impurity.

Method Validation for the Determination of Assay and Stability Indication of Rilpivirine by LC has been developed. In linearity study, the graphical representation of data proves that Rilpivirine and linearity in the range of 0.020 mg/ml to 0.030 mg/ml with $r^2$ value 0.9999. In system precision inject the standard solution (0.10 mg/mL in the diluent six injections and determine the percentage relative standard deviation, the % RSD for Rilpivirine was found to be 1.05 at the working concentration. The % RSD observed on the replicate indicates the precision of the system. In method precision study, was performed by analysing a sample solution of Rilpivirine at working concentration and the percentage relative standard deviation is 0.28.

In intermediate or inter-day precision study, the mean % drug content for Rilpivirine found to be 99.7 and 100.2 respectively. The % RSD for Temazepam found to be 0.21 and 0.28 respectively. There is no significant difference by same analyst by different instruments on different day. Therefore the intermediate or inter-day precision of the method can be considered to be acceptable and the bias
in assay determination for each parameter is less than \( \pm 1.5 \). The \% of recovery values were in the range of 98.7 – 100.5 and the percentage relative standard deviation for recoveries obtained was in the range of 0.35 – 0.50. Sample solution spiked with impurities was found to be stable up to 1600 minutes at 2-8°C. In robustness or system suitability study, there was no significant impact on the \% RSD and tailing factor. The results of the robustness study also indicated that the method is robust and is unaffected by small variations in the chromatographic conditions. In order to assess the stability indicating nature of the HPLC method, Rilpivirine samples were stressed by acid, base, hydrogen peroxide, heat and UV radiation. The degraded samples are analyzed using a photodiode-array detector.

The method enabled for RP-HPLC method was found to be simple, rapid, cost-effective, linear, accurate, precise and robust over the specified range; and selective for Rilpivirine without any interference from other components or additives. This method can be employed conveniently, reliably and successfully for the estimation of Rilpivirine for routine quality control and stability studies.

Method Validation for the Determination of Assay and Stability Indication of Artemether by Liquid Chromatography was studied. Six replicate injections of standard solution of Artemether were injected into the HPLC system and analyzed as per the proposed method. The areas of response of the analyst along with \% RSD are 0.06. The \% RSD observed on the replicates indicates the reproducibility and hence the precision of the system. Six samples of Artemether analyzed as per the method. Each named impurity and total impurities were calculated on these replicates. The \% RSD observed 0.18. These results comply with the acceptance criteria and indicating acceptable precision of the system. The \% RSD observed in the calculation of known impurities and total impurities indicate the precision of the method.

The linearity of the HPLC method was demonstrated for Artemether related substances solutions ranging from LOQ 20\%, 40\%, 80\%, 100\%, 120\% and 150\%. The linearity results for Artemether and impurities in the specified concentration range were found satisfactory, with a correlation coefficient greater than 0.99. System suitability followed by a sample analysis was run to assess if these changes had a significant effect on the chromatography. The number of
theoretical plates for Artemether peak is not less than 3000. The resolution between the peaks due to intermediate and Artemether not less than 2.0. The tailing factor for Artemether peak not more than 2.0. Theoretical plates for Artemether peak from first chromatogram of standard should be not less than 3000. Tailing factor for Artemether peak from first chromatogram of standard not more than 2.0 and % RSD for replicate standard injections not more than 5.0. Artemether and its impurities were analyzed individually and verified that the retention times is optimum.

The newly developed RP-HPLC method was found to be simple, rapid, cost-effective, linear, accurate, precise and robust over the specified range; and selective for Artemether without any interference from other components or additives. This method can be employed conveniently, reliably and successfully for the estimation of Artemether for routine quality control and stability studies.

Developed and optimised the Novel Method Developed Ilaprazole by Ultra Performance Liquid Chromatography (UPLC). Reversed phase UPLC was proposed as a suitable method for ilaprazole. Chromatographic system consisted of an Aquity UPLC BEH Shield RP 18 (100 X 2.1 mm), 1.7 μm and detector of UV at 237 nm and 210 nm. 1.40 μL as an Injection volume. The Precision of the method was determined by analyzing a sample of Ilaprazole solution spiked with impurities at 100% of the specification limit of six replicate sample preparations. The percentage relative standard deviation of recovery obtained for each impurity less than or equal to 5.0. Prior to this, system suitability parameters were calculated by injecting the system suitability solution. The % RSD was found to be 3.01. Percentage of recovery obtained is in the range of 100.4 – 100.6 at 80.0% to 120.0%. The linearity results for Ilaprazole and impurities in the specified concentration range were found satisfactory, with a correlation coefficient greater than 0.99. The limit of detection values obtained for each impurity was within the acceptance criteria. Signal to noise ratio about 3:1 and the detection less than 0.15%. Limit of Quantification (LOQ) values were determined from the same experiment as mentioned in the limit of detection section. The LOQ values obtained are presented in Table 5.7. Signal to noise ratio is about 10:1 and the quantification limit is to be less than level of specification preferably much less.
System suitability followed by a sample analysis was run to assess if these changes had a significant effect on the chromatography. A sample of Ilaprazole spiked with known impurities was analyzed for verifying the level of impurities at each variation. The retention time of all the impurities including Ilaprazole were affected by slight variation in the flow, pH and column temperature, however the system suitability criteria for the method were fulfilled. The number of theoretical plates for Ilaprazole peak not less than 3000. The resolution between the peaks due to intermediate and Ilaprazole are not less than 2.0. The tailing factor for Ilaprazole peak is not more than 2.0. No significant variation in the percentage of impurities was observed up to 48 hours at 2-8° C for reference solution and sample solution. The level of unknown impurity was found to increase in the sample solution stored at room temperature. It is recommended to keep the solutions at 2-8° C for analysis. Record the results and assign the stability of the solution based on the experimental data. For a stable solution, the individual impurity values to be within ±0.03 of the original value and the total impurities to be within ±0.10 of the original value.

An ultra performance liquid chromatography method was developed and validated for the determination of the related substances for ilaprazole. The proposed method was found to be good results for specificity, linearity, precision, intermediate precision and accuracy, stability in analytical solution, robustness and degradation studies. Therefore the method is suitable for its intended use for commercialization.

Future Outlook

Analytical techniques typically used to increase the analyst's confidence in the purity of chromatographic peaks include orthogonal separations, selectivity testing using potential impurities and fraction collections followed by alternate testing. Based on these studies a wide variety of drugs of forensic interest could be analyzed with superior resolution and speed over previously reported methodology using a C18 stationary phase by replacing the heptanesulfonate counter ion with methanesulfonate and using two isocratic mobile phases at different methanol concentrations. The use of smaller size columns and mobile increases HPLC's performance. Another way of increasing this technique's
performance is to increase the specificity of detection. This is important in both qualitative and quantitative analysis. For HPLC the relatively low peak capacity means that the reliability of compound identification by retention time only (even for screening purposes) is poor. In order to enhance the specificity of electrochemical, PDA or TSP-MS detection for certain compounds, post-elution photo irradiation was investigated. The capillary SFC generated much interest and excitement as a possible alternative technique to HPLC.

Since its discovery, HPLC has undergone sea changes with respect to its technical capabilities. In this direction the latest development has given rise to an improved version called Ultra High Performance Liquid Chromatography (UHPLC). The whole idea of introducing the UHPLC systems is to reduce analysis time considerably without compromising on performance. UHPLC increases throughput as well as decreased flow rates. There are varieties of mass analysers available today with varied analytical capabilities such as mass range and resolution. The most basic analysers will be the quadruple mass spectrometers. But the range and resolution must further be increased to cope with the existing conditions.