Chapter 6

DISCUSSION

6.1. Method Development, Validation and results of forced degradation studies of Armodafinil.

Armodafinil is a wakefulness promoting and a narcoleptic agent act by inhibiting the dopamine reuptake leading to increased dopamine levels. This drug was chosen as one of the analyte in the present investigation owing to the lack of literature methods for HPLC based stability indicating assay. Hence, due to the wide acceptability of RP-HPLC method for stability studies and in relation to the structure and physical properties of Armodafinil, RP mode was preferred. C8 column was chosen based on the requirement viz high hydrophobicity to retain the molecule structure and also based on wide acceptability in industrial application.

RP-HPLC method developed was found to be precise, accurate and robust for the estimation of Armodafinil and also for forced degradation studies. The method was developed by altering mobile phase composition, pH, buffers and their concentration. Finally the method was optimised with 55:45 v/v of water and methanol (10% OPA; pH 3). 10% OPA was preferred in the present method due to the polar nature of analyte, and to obtain adequate retention of the analyte. The pH was optimized to 3.0 and the retention time of the drug was 8.2 min. The drug in different concentrations was injected to predict the linearity range for Armodafinil. It was observed that Armodafinil obeys Beer Lamberts law in the range of 10 to 150µg/ml. The correlation coefficient was 0.9994. Recovery studies were performed at three levels i.e. 80%, 100% and 120% of test drug concentration and it was found to be in the acceptable range of 99.37-100.3%. Repeatability and intermediate precision were performed and it was in acceptable range with % RSD of < 2%. Robustness was determined by making deliberate changes in the method conditions and assay was performed. Specificity was performed by subjecting the drug to various stress conditions and was found to be specific.

The drug was subjected to various stress conditions like acid hydrolysis, base hydrolysis, neutral hydrolysis, oxidative degradation, thermal degradation and photo-degradation. It was observed that six major degradation products were formed with retention times of 4.4 min (D1), 6.6 min (D2), 9.8min (D3), 10.4min (D4), 13.3 min
(D5) and 15.7 min (D6) respectively. In acid stress studies four degradants were formed (D3, D4, D5, D6), base stress studies- one degradant (D1), photolytic stress studies- one degradant (D6), oxidative stress studies- one degradant (D2), thermal stress studies- one degradant (D4). Out of 6 degradants D1, D2, D4 are specific to base, oxidation, thermal respectively. D6 is the common degradant in both acid and photolytic stress studies. The resolution among all peaks was significant and was found to be more than 2.

6.2. Method Development, Validation and results of forced degradation studies of Sofosbuvir.

Sofosbuvir is a currently available antiviral agent that is used in chronic hepatitis treatment. It is a nucleotide analog inhibitor, which specifically inhibits HCV NS5B (non-structural protein 5B) RNA-dependent RNA polymerase. Lack of literature provoked us to choose the drug for the present investigation using RP-HPLC. Owing to its structure and physical properties RP mode was preferred. C18 column was chosen based on the requirement viz high hydrophobicity to retain the molecule structure and also based on wide acceptability in industrial application.

A RP-HPLC method was developed which was precise, accurate and robust for the estimation of Sofosbuvir and also for forced degradation studies. The method was developed by altering the mobile phase composition, pH, buffers and their concentration. Finally the method was optimised with 40:60 v/v of acetonitrile and water (10% OPA; pH 5.5). The 10% OPA was preferred due to the polar nature of analyte, and to obtain adequate retention of the analyte. The pH was optimized to 5.5 and the retention time of the drug was 8.9 min. Drug in different concentrations were injected to predict the linearity range for Sofosbuvir. It was observed that Sofosbuvir has shown an increase in the absorbance with concentration from 10, 15, 20, 25, 30µg/ml. The correlation coefficient was 0.9991. Recovery studies were performed at three levels i.e. 80%, 100% and 120% of test drug concentration and it was found to be in the acceptable range of 98-102 %. Repeatability and intermediate precision were performed and it was in acceptable range with % RSD of < 2%. Robustness was determined by making deliberate changes in the method conditions and assay was performed. Specificity was performed by subjecting the drug to various stress conditions and was found to be specific.
Posing the drug to various stress conditions like acid, base and neutral hydrolysis, oxidative, thermal and photo-degradation a total of 8 degradants were observed in the present forced degradation study for SBVR. PDA detector response for the peak purity of SBVR was more than 0.9985 in all stress conditions investigated. A total of 8 degradants (D1 – D8) were detected in the present study with retention time of 2.9 min (D1), 3.3 min (D2), 3.4 min (D3), 3.6 min (D4), 3.8 min (D5), 4.6 min (D6), 4.9 min (D7) and 6.9 min (D8). Degradants D1, D3, D4, D7 were formed in all stress conditions. D2 was specific to thermal and sunlight. D6 was specific to acid and photolytic stress. D5 and D8 were formed in all stress conditions except in hydrogen peroxide. It is susceptible to all other stress conditions.

6.3. Method Development, Validation and results of forced degradation studies of Tofacitinib citrate.

Tofacitinib is a januskinase (JAK) inhibitor used in the treatment of rheumatoid arthritis (RA) psoriasis, inflammatory bowel disease and other immunological diseases with the pKa of 8.4. The drug was chosen as one of the analyte in the present investigation owing to the lack of literature methods for HPLC based stability indicating assay. Hence, due to the wide acceptability of RP-HPLC method for stability studies and in concern to its structure and physical properties of TFB, RP mode was preferred. C18 column was chosen based on the requirement such as high hydrophobicity and to retain the molecule structure.

A novel RP-HPLC method was developed that was precise, accurate and robust for the estimation of Tofacitinib and also for forced degradation studies. The method was developed by altering mobile phase composition, pH, buffers and their concentration. Finally the method was optimised with 50:50 v/v of water and methanol and the retention time of the drug was 3.9 min. Drug in different concentration ranges were injected to predict the linearity range for Tofacitinib. It was observed that Tofacitinib obeyed linearity from 10-60µg/ml. The correlation coefficient was 0.9991. Recovery studies were performed at three levels i.e. 50%, 100% and 150% of test drug concentration and it was found to be in the acceptable range of 98-101.41 %. Repeatability and intermediate precision were performed and it was in the acceptable range with % RSD of < 2%. Robustness was determined by making deliberate changes in the method conditions and assay was performed.
Specificity was performed by subjecting the drug to various stress conditions and was found to be specific.

Tofacitinib was found to slightly degrade in oxidative condition (30% v/v H₂O₂) and mild degradation was observed in thermal stress conditions too. TFB was found to be stable in all conditions. Assay studies were carried out for stress samples against Tofacitinib qualified working standard. The purity and assay of Tofacitinib was unaffected by degradation products and thus confirms the stability-indicating power of the developed method.

6.4. Method Development, Validation and results of forced degradation studies of Milnacipran.

Milnacipran is a currently available adrenergic agent and can be used as an antidepressant and also for the treatment of fibromyalgia. It is a selective nor epinephrine and serotonin reuptake inhibitor with the pKa of 9.3. This drug was chosen as one of the analyte in the present investigation owing to the lack of literature methods for HPLC based stability indicating assay. RP method and C₁₈ Column was used in the present study in order to retain the structure of the molecule.

A RP-HPLC method was developed which was precise, accurate and robust for the estimation of Milnacipran and also for forced degradation studies. The method was developed by altering mobile phase composition, pH, buffers and their concentration. Finally the method was optimised with 55:45 v/v of water and methanol and the retention time of the drug was 2.8 min. The drug at different concentrations was injected to predict the linearity range for Milnacipran. It was observed that Milnacipran was linear from 10-60µg/ml. The correlation coefficient was 0.9991. Recovery studies were performed at three levels i.e. 50%, 100% and 150% of test drug concentration and it was found to be in the acceptable range of 98-102 % . Repeatability and intermediate precision were performed and it was in acceptable range with % RSD of < 2%. Robustness was determined by making deliberate changes in the method conditions and assay was performed. Specificity was performed by subjecting the drug to various stress conditions and was found to be specific.

MIL was found to degrade significantly with peroxide and very mild degradation was observed in acid, base, photolytic, thermal and hydrolytic stress conditions
conditions. Photodiode array detector was employed to check and ensure the homogeneity and purity of MIL peak in all the stressed sample solutions. Assay studies were carried out for stress samples against MIL qualified working standard. The purity and assay of MIL was unaffected by the presence of its degradation products and thus confirms the stability-indicating power of the developed method.

6.5. Method Development, Validation and results of forced degradation studies of Leflunamide.

Leflunamide is an anti-rheumatic agent employed in treating rheumatoid and psoriatic arthritis. It is a pyrimidine synthesis inhibitor, with the pKa of 10.41. The drug was chosen as one of the analyte in the present investigation owing to the lack of literature methods for HPLC based stability indicating assay. Hence, due to the wide acceptability of RP-HPLC method for stability studies and in concern to its structure and physical properties of LFN, RP mode was preferred. C18 column was chosen based on requirement of high hydrophobicity to retain the molecule structure and also based on wide acceptability in industrial application.

The RP-HPLC method developed was precise, accurate and robust for the estimation of Leflunamide and also for forced degradation studies. The method was developed by altering mobile phase composition, pH, buffers and their concentration. Finally the method was optimised with 70:30 v/v of methanol and water and the retention time of the drug was 1.06 min. The drug in different concentrations was injected to predict the linearity range for Leflunamide. It was observed that Leflunamide was linear from 10-60µg/ml. The correlation coefficient was 0.9991. Recovery studies were performed at three levels i.e. 50%, 100% and 150% of test drug concentration and it was found to be in the acceptable range of 98-102 %. Repeatability and intermediate precision were performed and it was in acceptable range with % RSD of < 2%. Robustness was determined by making deliberate changes in the method conditions and assay was performed. Specificity was performed by subjecting the drug to various stress conditions and was found to be specific.

The drug was subjected to various stress conditions like acid hydrolysis, base hydrolysis, oxidative degradation, thermal degradation and photo-degradation. The degradations are observed in all stress conditions. Slight degradation was observed in
oxidative and photolytic stress conditions. In acid, base and thermal stress conditions wild degradants are formed.