*Res.J.Chem.Sci.*_____*Research Journal of Chemical Sciences*
Vol. **1(4),** 74-79, July **(2011)** ISSN 2231-606X

# Stress Degradation Studies and Development of Validated Stability Indicating Method for Assay of Mirtazapine

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**Available online at: www.isca.in**
(Received 15th May 2011, revised 30th May 2011, accepted 09th June 2011)

## Abstract

*This study describes the development and validation of stability indicating RP-HPLC method for Mirtazapine, an antidepressant drug. In order to investigate the stability of drug, a stress testing of drug sample by exposing it to variety of forced degradation conditions has been recommended. Mirtazapine was subjected to stress degradation[1] under different conditions recommended by International conference on Harmonization (ICH). The ICH guideline gives parameters to be considered when validating methods, the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. Stress testing methods are screening methods to be used to understand the degradation chemistry of a drug and therefore do not need to be validate to the extent of final control methods. The sample so generated was used to develop a stability indicating High Performance liquid Chromatographic method for Mirtazapine. The chromatographic separation of Mirtazapine and its degradation products was done on C18 column. The mobile phase containing mixture of Water and Acetonitrile in ratio 80:20 was found to be most satisfactory at a flow rate of 1mllmin. Detection was carried out using single wavelength detector at 225nm. The retention time under optimized chromatographic condition was found to be 8.43 minutes, with asymmetry of 1.50. A good linear response was observed in the range of 5-25ug/ml. The method showed good recoveries (average 101.37).*

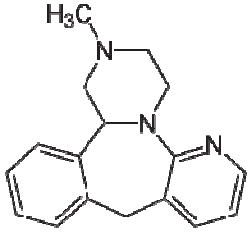
**Key words:** RP-HPLC, stability indicating, Mirtazapine.

## Introduction

The advancements in the field of drug discovery during recent years have introduced several new drug molecules belonging to various pharmacological categories. The safety and efficacy of these drug formulations is of utmost importance. The drugs may get contaminated with process impurities during their formulation. They may also undergo degradation under environmental conditions leading to contamination with degradation products thereby lowering / altering the therapeutic efficacy and safety. Drug regulatory authorities around the world require that impurities in drug substance and drug product when present at threshold levels recommended by the International Conference on Harmonisation (ICH) be isolated and characterized. Degradation product is a molecule resulting from a change in the drug substance brought about over time which could occur as a result of processing or storage. The term drug stability refers to extent to which drug substance or product retain, within specified limit throughout its period storage and having same properties and characteristics those were present at the time of manufacturing. According to US FDA guidelines Stability Indicating Method (SIM) is defined as validated analytical procedure that accurately and precisely measure Active Pharmaceutical Ingredient (API)

free from potential interferences like degradation products, process impurities, and other potential impurities[1]. The probable degradation product of API can be produced by certain stress conditions like hydrolytic (acidic, basic, etc), oxidative, thermal, photolytic, etc. The aim of this work was to develop SIM for determination of Mirtazapine in presence of its degradation products. In the present study, stress degradation[2] of Mirtazapine was effected by hydrolysis under acidic and basic conditions, oxidation with $H_2O_2$, dry heat degradation, and photo degradation.

**Structure of Mirtazapine:** Mirtazapine, chemically is 1,2,3,4,10,14b-Hexahydro-2-methyl pyrazino[2,1-a]pyrido [2,3-C][2]benzazepine. It is used as an antidepressant for the treatment of moderate to severe depression. It is classified as a noradrenergic and specific serotonergic antidepressant (NaSSA).




It is official in USP[3] and HPLC method has been prescribed for bulk drug and tablet formulation. HPLC assay with fluorescence detection for the routine monitoring of Mirtazapine (and its demethyl metabolite) in human plasma is reported[4,5] Determination of Mirtazapine in human plasma by liquid chromatography is also reported[6]. No useful method for the analysis of Mirtazapine in presence of its degradation products is yet reported.

## Material and Methods

Mirtazapine was kindly provided by Sun Pharmaceuticals Industries Ltd, Mumbai, India. All the chemicals and solvents used were of AR or HPLC grade and Whatman filter paper no 42 was used throughout the experimental work. Mirtazapine tablets were procured from a local pharmacy. Younglin HPLC, ACME 9000 comprising of SP 930 D pump, single wavelength detector and Rheodyne injector fitted with 20 microlitre capacity loop was used in the study. Separation and quantitation was done using Reverse phase varian C-18 Neosphere (250 x 4.6mm) column.

**Chromatographic Conditions:** The mobile phase was prepared by mixing Water (HPLC grade) and Acetonitrile in ratio 80:20. In a 100ml volumetric flask take 0.3% (0.3ml) Tri ethyl amine and adjust volume with water up-to 100 ml, adjust pH to 3. Take 80 ml of this and add 20 ml of Acetonitrile. The mobile phase was filtered and degassed by ultrasonic vibrations prior to use. The flow rate was 1mllmin. All determinations were performed at ambient temperature. An accurately weighed sample (10mg) of Mirtazapine was transferred to a 10 ml volumetric flask and dissolved in methanol to obtain a solution of strength 1000 ug/ml. One milliliter of this solution was then transferred in 10ml volumetric flask and volume was made up-to the mark with mobile phase. This gave the standard stock solution of 100 ug/ml (A). Take 1ml from A in 10ml volumetric flask and dilute with mobile phase upto the mark: 10 ug/ml (B).

**Study of System Suitability Parameters:** The chromatographic conditions were set and five replicate injections were made separately and the chromatograms were recorded. One of the standard chromatogram is depicted in figure:1.

**RP-HPLC Assay procedure:** Twenty tablets, each containing7.5mg Mirtazapine[7] were weighed and finely powdered. A quantity of powder equivalent to 83.98 mg was weighed and transferred in 25 ml volumetric flask and volume was made upto 25 ml with methanol. The solution was filtered. From the filtrate appropriate dilutions were made in mobile phase. The tablet sample solution was injected and chromatogram was obtained. The peak area of the Mirtazapine was calculated. Using the regression equations and peak areas of the sample, the amount of Mirtazapine in the sample was calculated. The amount of Mirtazapine per tablet was thus found.

**Method Validation: Linearity of Response:** Aliquot portions of standard stock solution were diluted with methanol and subsequently with mobile phase. (conc.: 5-25ug/ml). The chromatographic conditions were set and standard solutions of different concentration were injected separately and the chromatograms were recorded. A graph was plotted as peak area vs. concentration of drug and is depicted in figure 2 below:

**Accuracy:** Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method. The recovery studies were carried out at levels of 70%, 85%, 100%, 115%, and 130% and percent recovery was calculated.

**Precision:** Precision of analytical method is expressed as SD and RSD of series of replicate measurements (S.D. – 1.34, % RSD – 1.32)

**Intermediate Precision:** The intermediate precision was done according to ICH guidelines by using three standard concentrations and the results are given in table 1.

| Concentration | Inter-day | Intra-day | Mean | Std Dev (SD) | % RSD |
|---|---|---|---|---|---|
| 2 | 2.17 | 2.19 | 2.19 | 0.014 | 0.6422 |
| 10 | 9.92 | 9.93 | 9.93 | 0.0001 | 0.001 |
| 20 | 19.56 | 19.5 | 19.5 | 0.0433 | 0.2217 |

**Ruggedness:** The studies were carried out for intra-day and inter day and results are shown in table 1.

**Robustness:** The sample was analyzed using proposed method after a deliberate change in detection wavelength for estimation by +-2nm.

**Forced degradation (stress studies) of Mirtazapine:** The stress studies were performed by using 1mglml methanolic solution of Mirtazapine and applying it to various stress conditions to study the effect over wide range of pH, heat, oxidation, and photo degradation using the following approach:

**Acid Degradation**: 50 mg of Mirtazapine was dissolved in 50ml of 0.1N methanolic hydrochloric acid (1mg/ml) and 25 ml of it was refluxed in round bottom flask on boiling water bath for 8 hr. The remaining solution was kept at room temperature.

**Alkali Degradation**: 50 mg of Mirtazapine was dissolved in 50 ml of 0.1 N methanolic sodium hydroxide (1mg/ml) and 25 of it was refluxed in round bottom flask on boiling water bath for 8hr.The remaining solution was kept at room temperature.

**Oxidative Degradation**: 50 mg of Mirtazapine was dissolved in 50 ml 3% $H_2O_2$ (1mg/lml) and 25 ml of the above solution refluxed in round bottom flask on boiling water bath for 8hr.The remaining solution was kept at room temperature.

**Photo Degradation**: Ten mg of samples of Mirtazapine, evenly spread in thin layer in a covered Petridish were kept in sunlight for different time intervals and also in dark as a blank.

**Thermal Degradation**: Ten mg samples of Mirtazapine in different weighing bottles were kept at 70˚C and 25˚C for different time intervals.

**Sample Preparation and HPLC Resolution:** The samples (1ml each) were withdrawn during stress studies (1-3) above every $1^{st}$,$3^{rd}$,$5^{th}$ and $8^{th}$ hour to study the extent of degradation and to stop forced degradation after obtaining degradation of about 20%.To compare the effect of various stress conditions at elevated temperature and R.T. corresponding blanks were kept at R.T. and the samples were withdrawn simultaneously. The withdrawn samples were diluted to 10 ml with methanol (the samples of acid and alkali degradation were neutralized prior to dilution).The resultant solutions (1ml each) were further diluted to 10 ml with mobile phase.

In case of thermal degradation and photo-degradation studies the samples were withdrawn every $7^{th}$, $14^{th}$, and $30^{th}$ day. In all these cases the total quantity of each withdrawn sample was dissolved in methanol and diluted to 10 ml with methanol, the resultant solution (1ml each) was further diluted to 10 ml with mobile phase.
The stressed samples so prepared were injected in HPLC column and chromatograms were obtained using preliminary optimized mobile phase. The chromatograms of degradation products are depicted.

## Results and Discussion

The percentage of drug found in formulation was 100.18%. The result of analysis shows that drug was in good agreement with the label claim of the formulation. The data obtained in the calibration experiments when subjected to linear – regression analysis showed a linear relationship between peak areas and concentrations in the range of 5 – 25 ug/ml for Mirtazapine. The developed method was found to be precise as replicate estimation of Mirtazapine in tablet were analyzed by proposed method and have yielded quite concurrent results, speaks about repeatability of the method. Good recoveries 101.37% of the drug indicates that the method was accurate.

The mobile phase containing mixture of water and Acetonitrile was found to be most satisfactory as it gave good resolution of drug and degradation products with reasonably symmetrical sharp peaks. A detection wavelength 225 was optimized as Mirtazapine has maximum high absorbance at this wavelength. A flow rate of 1mllmin with normal temperature was found to be optimum. The retention times under optimized chromatographic conditions was found to be 8.33 min with asymmetry of 1.18. The degradation products of Mirtazapine were well resolved. The high capacity factor values 2.74 indicate good retention of the drug. Almost all degradants were well resolved and eluted prior to parent drug. The total run time of chromatogram was about 10 min. The retention time of degradants were 2.48, 5.1, and 11.65 (acid stress degradant) 2.6 min, and 6.8 min (alkaline stress degradant) 5.8, 8.8 and 10.5 (oxidative stress degradant) 5.1min (photolytic degradant). The observed number of theoretical plates per meter (6016.1) indicates substantially high column efficiency.

The detector response was found to be linear over the concentration range 5-25 ug/ml. The results of estimation by proposed method on different days were very much reproducible. During robustness check the variation in detection wavelength by +-2nm has not any significant change in results, however the mobile phase composition was found to be quite critical and a small change in the proportion of mobile phase has resulted in the loss of resolution of the degradants.

The results of assay of Mirtazapine tablet obtained by proposed HPLC method are quite concurrent and reproducible. The recovery of the drug from the tablet was about 100% indicating accuracy and reliability of method and non interference of Excipients. At the same time method is simple, rapid, reasonably specific and rugged. Mirtazapine drug was found to degrade under acidic condition. When Mirtazapine was treated with 0.1 N Hcl and sample was withdrawn after an interval 1, 3, 5, and 8 hours the drug undergo degradation and the typical chromatogram obtained after 1 hour is shown in figure 3. The degradation reaction was more intense and quicker in alkaline condition. When Mirtazapine was treated with 0.1N NaOH and sample was withdrawn after 1, 3, 5 and 8 hours, it undergoes degradation and typical chromatogram obtained after 1 hour is shown figure 4. Upon treatment of Mirtazapine with 3% $H_2O_2$ at normal condition no additional peaks were detected but after refluxing it, it undergoes degradation figure 5. When Mirtazapine was exposed to light source, the Mirtazapine content exhibited slight decrease, and an additional peak was also detected. Figure 6 shows the chromatogram of Mirtazapine degraded under Photolytic condition. When Mirtazapine was exposed to heat there was no change in the peak area for Mirtazapine. No

additional degradation peaks were detected. While figure 7 shows the chromatogram of Mirtazapine exposed to dry heat, figure 8 shows the chromatogram of Mirtazapine tablets. When the marketed tablets without primary packaging were subjected to milder stress conditions there was no peak for product of degradation.

## Conclusion

Thus the study shows that Mirtazapine undergoes degradation in acidic, alkaline, oxidative and photolytic conditions whereas it is relatively stable under dry heat condition for the time it was exposed. The method proved to be simple, accurate, precise specific and selective. Hence it may be used for stability studies

## Acknowledgements

The authors wish to express their gratitude to Sun Pharmaceutical Industries Ltd., Mumbai, India for providing gift sample of Mirtazapine. The authors are also thankful to Dr. K. P. Bhusari, Principal Sharad Pawar college of Pharmacy for providing necessary facilities to carry out the research work.

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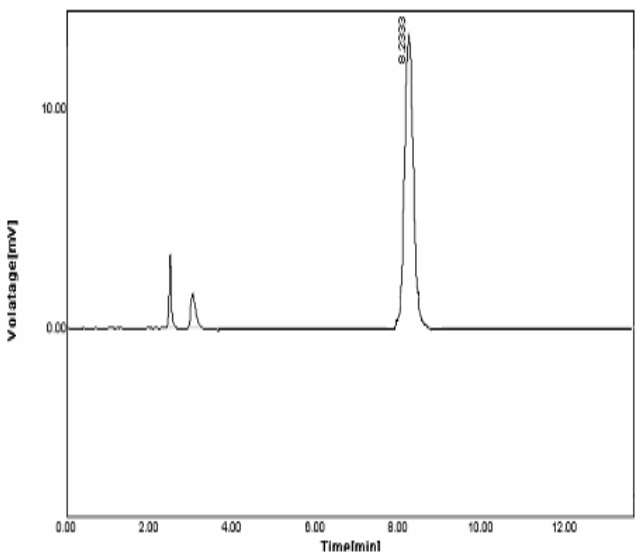


**Figure-1: HPLC Chromatogram of Mirtazapine**

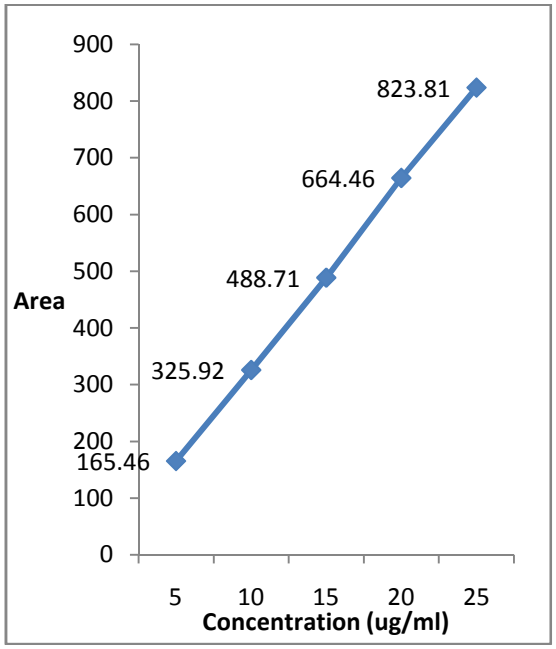


**Figure-2: Linearity of Response**

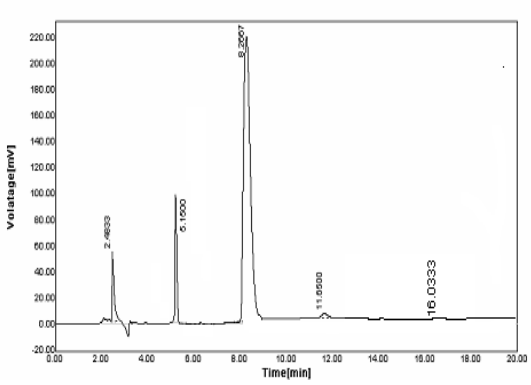


**Figure-3: Acid Degradation**

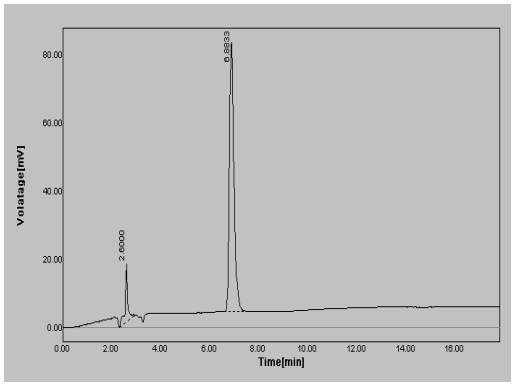


**Figure-4: Alkali Degradation**

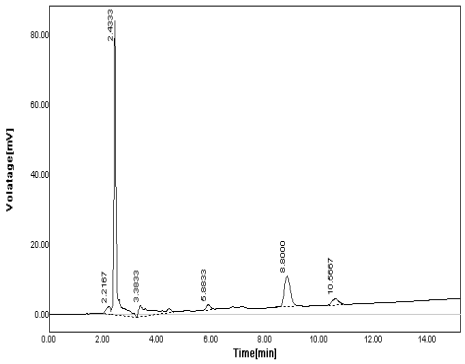


**Figure-5: $H_2O_2$ Degradation**

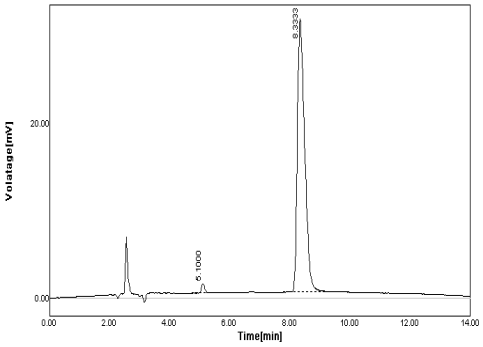


**Figure 6: Photo Degradation**

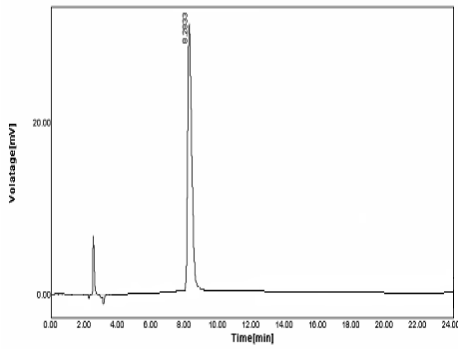


**Figure 7: Thermal Degradation**

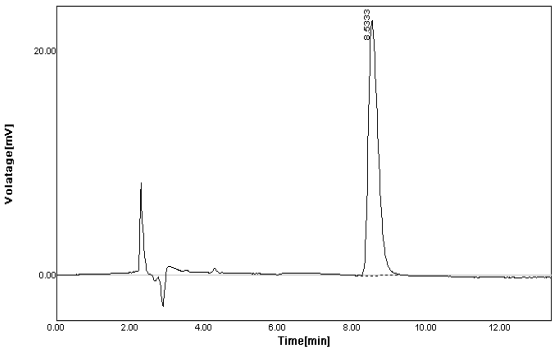


**Figure-8: Tablets**