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(RESEARCH ARTICLE)

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Method development and validation for the estimation of mirtazapine by using UV spectrophotometer with different order of derivatives

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Abstract

A precise, accurate, effortless, and inexpensive UV spectrophotometric was developed and authenticated for the appropriate estimation of Mirtazapine in bulk and marketed formulation. The solution consisting of Mirtazapine was being scanned over by UV- visible rays, ranging from its maximum wavelength absorbance. The numerous calibrations of standard Mirtazapine were formulated, and the absorbance for each was documented in response to the maximum wavelength. A graph was then plotted with the documented values of Concentration with that of the absorbance. Owing to this graph, the range and linearity were calculated. The varied analytical frameworks namely, accuracy, precision, LOD, and the LOQ were being enumerated, by the means of QC control. The derivative spectrophotometric method was established, which also authenticates all the parameters in consideration. The First to the Fourth Order Derivative of spectrophotometric was being computed, in the specified ranges of 200 nm to 400 nm. At around 222 nm, a sharp peak was observed. The Calibration Curve was plotted against concentration vs. absorbance. The selected maximum wavelength was considered 222 nm. A separate study was being conducted to test the accuracy and precision of the method, and the results generated were quite satisfactory. The drug consisting of 50 %, 75%, and 100% of – had appreciable recoveries within the range of 98 to 100%. These results claim that the method is safe and accurate. The Limit of Detection (LOD) and the Limit of Quantitation (LOQ) were also computed for the method. This method is also certified by the International Conference of Harmonization (ICH). All the validation parameters were within acceptable ranges. Thus, this method was determined and was used for successful application in the estimation of the amount of Mirtazapine present in pharmaceutical formulations.

Keywords: UV Spectroscopy; Analysis; Linearity; %Recovery; Derivative; Precision; Mirtazapine

1. Introduction

Depression is a fatal and life-threatening condition, which does not differentiate anyone based on their age or one's economic status. It is as likely to attack a child, as it is to a 60-year-old man. It comes along with the baggage of unprecedented and unimaginable pain. It causes absolute disruption and disarrangement in insignificant life chores. It disturbs a person to the core, and coming out of it is a battle in itself. Some of the most commonly observed and equally disruptive symptoms of this psychopathological disease include low or depressed mood, dysthymia, and lack of energy or fatigue. It is an extremely complicated situation consisting of various permutations of different and multiple etiologies and not just one sickness. [1] Mirtazapine is one such drug, chemically known as 1, 2, 3, 4, 10, 14b-Hexahydro-2-methyl pyrazino [2,1a] pyrido [2, 3-C] benzazepine. The empirical formula of the Mirtazapine is $C_{17}H_{19}N_3$ and the Molecular mass is 265.360 g·mol−1. [2] It is a specific serotonergic antidepressant (NaSSA) and noradrenergic that acts

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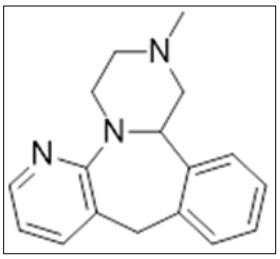
by antagonizing the adrenergic alpha2-autoreceptors and alpha2-heteroreceptors as well as by blocking 5-HT2 and 5-HT3 receptors. It enhances the release of5-HT1A-mediated serotonergic transmission and norepinephrine. [3] Mirtazapine is available on the market in tablets 7.5, 15, 30, and 45 mg doses. [4]The literature survey revealed that various methods for the determination of Mirtazapine in the plasma and pharmaceutical formulation have been developed. A rapid, sensitive HPLC-fluorescence method for the determination of Mirtazapine and Its two Major Metabolites in human plasma was reported in the literature.[5] Reversed-Phase HPLC Method has been also reported for separation and simultaneous determination of process-related substances of Mirtazapine in bulk drugs and formulations.[6] Development and validation of an LC-MS/MS method for quantitative analysis of Mirtazapine in human plasma were also reported.[7]Validation of a GC/MS method for the determination of alkaline drugs in whole blood was also reported.[8] Development and validation have been reported of a stability-indicating HPTLC method for the determination of Mirtazapine as a bulk drug and in pharmaceutical formulation. [9] A recent survey through the available literature implies the fact that only the zero-order, first-order, and the second-order derivatives are present [10], however, no data supporting the presence of third-order and fourth-order derivatives is found yet by the Spectrophotometric method for the estimation of Mirtazapine. This method can play a crucial role in ruling out solutions for all the problems linked with drug combination, drug impurities, and interference of excipients of drug an absorbance was found to be 222nm and the spectrum was scanned for the drug dissolved in methanol.



Mirtazapine

2. Material and methods

2.1. Materials and Apparatus

The Mirtazapine was kindly supplied as a gift sample by Sandoz Pvt. Ltd., Mumbai (India). Mitraz tablet (7.5 mg) of sun pharma purchased and all the rest of chemicals used for the HPLC grade were, A double beam UV-Visible spectrophotometer, (V-630 UV/ VIS spectrophotometer) having two cells of equal length of 1cm path. Light Source (Deuterium lamp 190 to 350 and Hydrogen lamp 330 to 110) while the light exchange Between 330 to 350. Detector Silicone photodiode (1337).

2.2. Preparation of Stock Solution

Mirtazapine (100 mg) was accurately weighed and transferred to a 100ml volumetric flask. It was first dissolved in 10ml of methanol and sonicated for about 10 min., then made up to the volume with water (100µg/ml) and finally filtered with Whatman filter ≠ 41.

2.3. Preparation of Sample Solution

Fresh aliquots from the standard stock solution were pipetted out and suitably diluted with water to get the final concentration in the range of 10-50µg/ml. The solutions were scanned under spectrum mode for 200-400nm wavelength range against blank (Table 1), and a sharp peak was obtained at 222 nm. A calibration curve was plotted taking an absorbance on the y-axis against the concentration of the standard solution on the x-axis (Fig. 1)

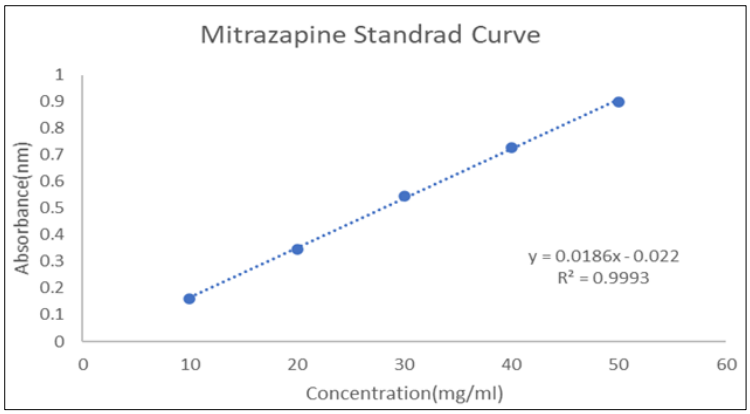


Figure 1 Calibration Curve of Mirtazapine

Table 1 Linearity of Mirtazapine

Sr.no	Concentration (µg/ml)	Absorbance
1	10	0.1598
2	20	0.3468
3	30	0.5473
4	40	0.7266
5	50	0.9001

2.4. Analysis of marketed formulation of Mirtazapine

For the analysis of the dosage form, 10 tablets of mirtazapine were weighed. The powder form, approximately equivalent to 10mg of Mirtazapine, was taken in a 100 ml volumetric flask. The formulation was first to be dissolved in 10ml of methanol and then sonicated for about 10 minutes. This solution was then filtered, the final dilution of this sample (10µg/ml) was obtained and the absorbance against blank at 222 nm was computed. The defined amount of Mirtazapine was calculated, with the help of the equation referring to the calibration curve (Table 2).

Table 2 Assay of Mirtazapine tablet

Dosage form	Label claim (7.5 mg)	Amount found* ±SD	% Purity of the tablets (% RSD)
MITRAZ	7.5	7.35±0.58	100.14±0.438

Table 3 Validation parameters of Mirtazapine

Parameters	Value
Absorbance (nm)	222 nm
Correlation coefficient	0.999
Molar extinction coefficient / ($L.mol^{-1}.cm^{-1}$)	5.76×10^4
Regression equation	y=0.086x-0.022
Correlation coefficient (r^2)	0.998
LOD/ (µg. mL^{1})	0.0260
LOQ/ (µg. mL^{1})	0.0875

3. Validation of methods

3.1. Linearity

Fresh aliquots were prepared from the stock solution (100µg/ml) ranging from 10-50µg/ml. The samples were scanned in a UV-Visible spectrophotometer using water as blank. It was found that the selected drug shows linearity between 10-50µg/ml (Table-3).

3.2. Accuracy

The precision and accuracy of the method are validated and verified by the virtue of the study of recovery which was being performed on 3 varied concentrations i.e. 50, 75, and 100 µg/ml of the expected, abiding by the ICH guidelines, through a replicate analysis (n=6). The standard drug solution was then mixed with a pre-analyzed sample solution and the percentage drug content was also computed. The results from the study of the accuracy of the drug were reported in Table 3. Recovery [(ct-cu)/ca] x100.

Here,

ct is the concentration of the analyte found.

cu is the total conc. of the analyte present in the formulation.

ca is the conc. of the pure analyte added to the formulation (Table-4).

Table 4 Accuracy range of Mirtazapine

Sample ID	Pure Drug (µg /ml)	% Recovery ±SD	%RSD
50%	5	98.89≠ 0.308	0.310
75%	7.5	100.19≠0.316	0.314
100%	10	100.21±0.270	0.262

3.3. Precision

The precision (intra-day precision) of this method was found by the Six independent test samples of Mirtazapine. The outcome t of the intermediate precision (inter-day precision) was evaluated with the help of 2 different analysts, on different days in different laboratories. The Percent Relative Standard Deviation (%RSD) values are enumerated (Table 5).

Table 5 Precision of Mirtazapine

Sample No.	(intra-day precision)	(intra-day precision)
1	0.224	0.214
2	0.224	0.215
3	0.226	0.215
4	0.227	0.215
5	0.225	0.214
6	0.223	0.220
Mean	0.226	0.2156
S. D	0.001	0.002
%RSD	0.66	0.91

3.4. Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of Detection (LOD) and Limit of Quantification (LOQ):

The LOD and LOQ were computed by the means of Standard Deviation of the response and slope of the calibration curve. (Table 3)

The formula for the LOD and LOQ are as follows: [11]

LOD = 3.3σ/s

LOQ = 10σ/s

4. Spectrophotometric derivative of different orders

Stock solution (1000 µg/ml) was prepared with 100mg standard drug of Mirtazapine Added in 100 ml of volumetric flask and dissolved in 10 ml of methanol makeup volume 100ml by water. Working on the standard stock solution of 20 µg/ml was used as a reference. The pharmaceutical tablet contains 7.5 mg of Mirtazapine

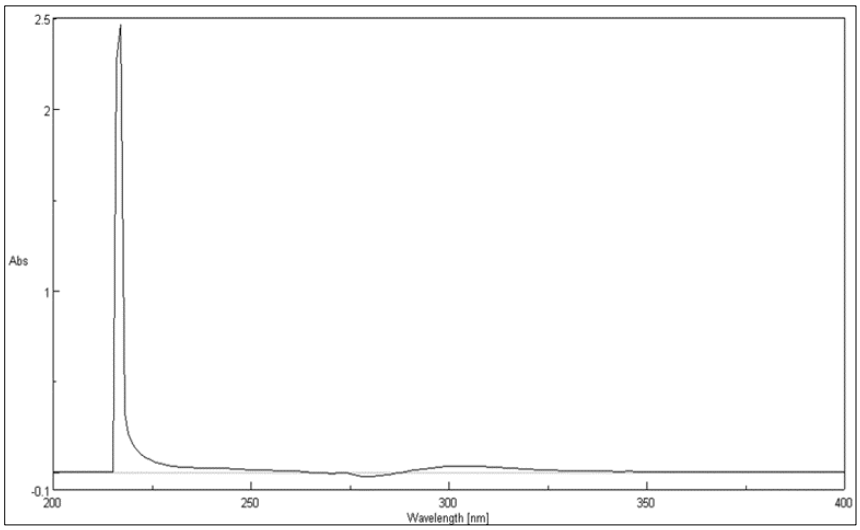


Figure 2 First Order derivate of Standard Mirtazapine

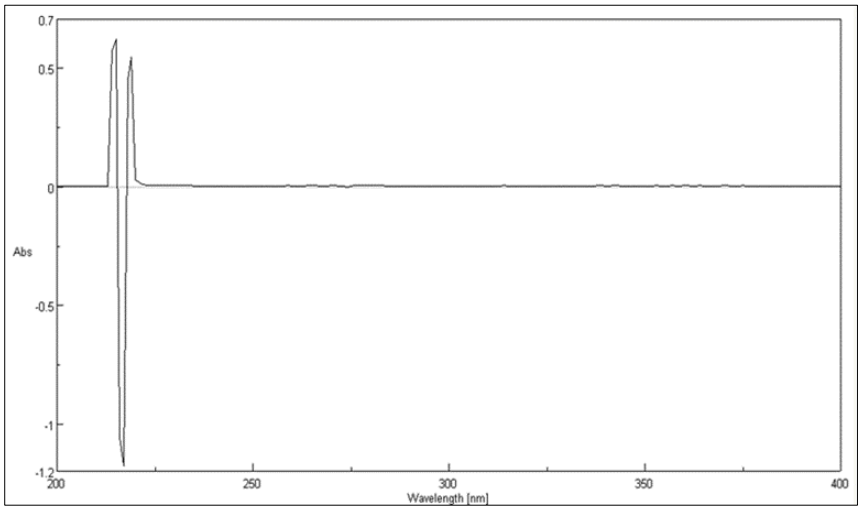


Figure 3 Second-Order derivate of Standard Mirtazapine

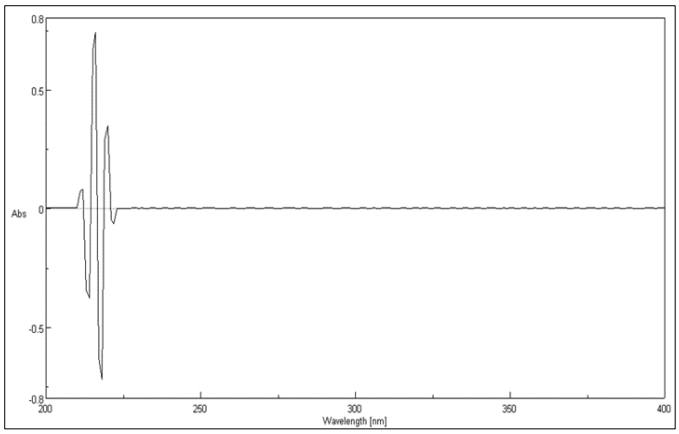


Figure 4 Third-order derivative of Standard Mirtazapine

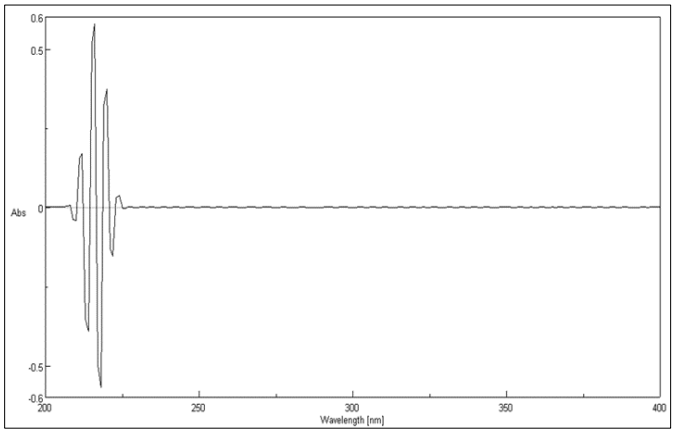


Figure 5 Fourth order derivate of Standard Mirtazapine

4.1. Procedure

The proposed method was applied marketed tablet Mitraz 7.5mg (Sun Pharma). Ten tablets of mirtazapine were weight accurately and powdered. the amount of Tablet power equivalent to 7.5 mg weight was accurately transferred to 50 ml of volumetric flask.10 ml of methanol was used for dissolved the drug then sonicated for 10 min and make up the volume by adding water after filtration with watmaan filter paper ≠ 41 proper dilutions were prepared at a range of 10-50 µg/ml with water UV spectra recorded with reference of water. Throughout the calibration curves, the average weight of one tablet was calculated.

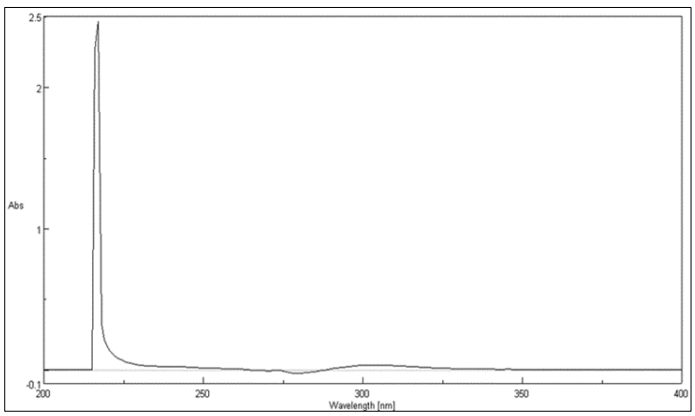


Figure 6 First order derivate of Mirtazapine tablet

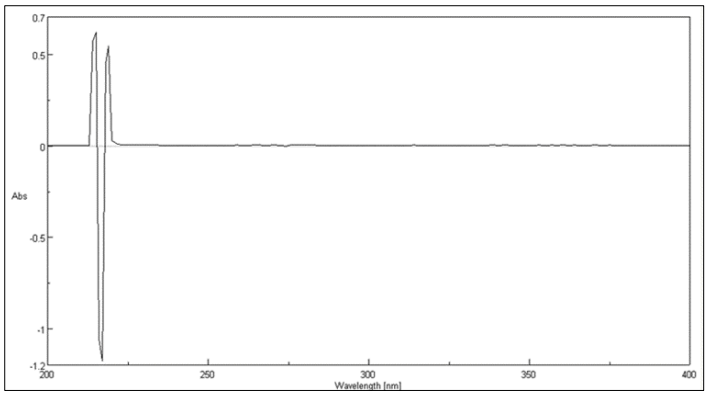


Figure 7 Second-order derivate of Mirtazapine tablet

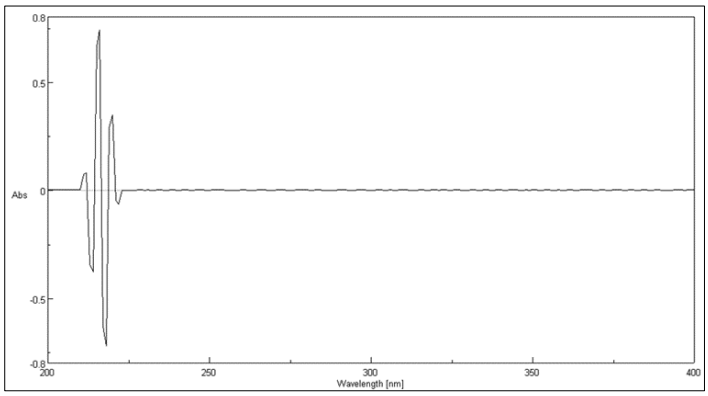


Figure 8 Third-order derivate of Mirtazapine Tablet

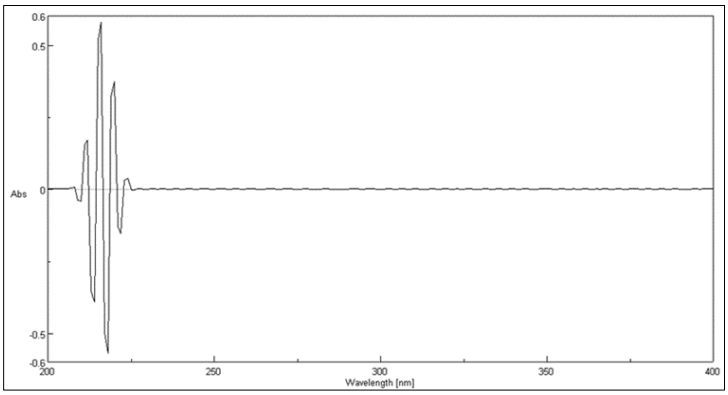


Figure 9 Fourth order derivative of Mirtazapine tablet

Table 6 Validation parameters of the developed method

Parameters	First-order	Second-order	Third-order	Fourth Order
Linear range (µg ml-1)	10-50	10-50	10-50	10-50
Determination coefficient	0.99995	0.99997	0.99982	0.99996
Correlation coefficients	0.99998	0.99997	0.99991	0.99993
Standard error of intercept	6.63 X 10-4	1.26 X 10-4	1.76 X 10-4	1.52 X 10-4
Regression equation	y= -0.004 + 0.008x	y= -0.007 + 0.024x	y = -0.006+ 0.002x	y = -0.009 + 0.003x

5. Result and discussion

The first order, second order, third order, and fourth-order, of standard Mirtazapine. Each spectrum is used for the determination of this drug. The first-order spectrum show well defined sharp peak at 222 nm and the second order, third order, and fourth-order show an opposite peak at 222 nm first to fourth derivatives order of UV spectra in water. No changes should observe in the maximum wavelength of all spectra. In this spectroscopic technique, a simple and specific model for determining Mirtazapine in tablet form is developed. All the solutions were scanned under spectrum mode at 200-400 nm wavelength range with a blank and sharp peak at 222 nm and all rest parameters are validated to get results between the ranges Table. 6. It should also be considered that all the other methods give similar and in some cases favorable results concerning their accuracy and precision. The methods thus proposed were found to be precise as % RSD values for intraday and Interday were satisfactory. The drug at each of the 50 %, 75 %, and 100 % levels showed remarkable recoveries that are in the range of 98 to 100%. All RSD values are less than 10% and the recovery as a measure of the accuracy is close to 100% in all the cases. These criteria were given in the literature for validation

6. Conclusion

From the results and discussion in this article Derivatives, spectrophotometric methods were developed and validated as per ICH guidelines Q2 (R1). In this paper, we discuss the determination of Mirtazapine in bulk as simple, sensitive, and reproducible forms. Solutions to all the problems linked with drug combination, drug impurities, and interference of excipients of drug an absorbance were found to be 222nm and the spectrum was scanned for the drug dissolved in methanol. The proposed methods can be successfully applied for Mirtazapine without any interference in quality control.

Compliance with ethical standards

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Disclosure of conflict of interest

The author has no conflicts of interest.

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Author's short biography

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Gaurav Manglaprasad Prajapati graduated from Pune University in 2020 with a bachelor's degree in pharmacy and is now progressing into M Pharmacy in department of Quality Assurance. He has also published numerous review articles in the Pharmaceutical sciences field. He is currently working on his final year project- stability indicating method development and validation of anticancer drug. He stands out to be an enthusiastic and keen learner. He believes in bringing out the best while working alone or in a group. He is keen on learning new things and grabbing new opportunities.