

## Original Research Article

# Development and Validation of UV- Spectroscopic method For Simultaneous Estimation of Metformin Hydrochloride and Pravastatin Sodium

### ABSTRACT:

**Introduction:** A new, simple, precise, accurate, and reproducible method was developed and validated for the simultaneous estimation of Metformin Hydrochloride (MH) and Pravastatin Sodium (PS) in pure form.

**Methodology:** Simultaneous estimation of Metformin Hydrochloride (MH) and Pravastatin Sodium (PS) was estimated by the ultraviolet (UV) spectrophotometry using absorbance subtraction method. The method was based on the measurement of absorbance at two wavelengths 232 nm and 238 nm, of metformin and Pravastatin Sodium respectively. These studies were performed at three different levels (75%, 100% and 125%) and the % recovery of MH and PS was calculated.

**Results:** The LOD and LOQ were found to be 0.481 µg/ml and 0.670µg/ml for MH and 1.15µg/ml and 1.68µg/ml for PS respectively. All the statically analyses were within the standard limits. It proves that the method was repeatable and selective for the simultaneous.

**Conclusion:** Therefore, the present study concludes that it can be successfully used for simultaneous estimation of MH and PS in pure and pharmaceutical dosage forms. The developed method was found to be simple, precise, and accurate.

*Keywords: Metformin Hydrochloride, Pravastatin Sodium, UV-visible spectroscopy, Simultaneous estimation, Absorbance subtraction method.*

## 1. INTRODUCTION

Chemically Metformin Hydrochloride (MH) is 1,1-Dimethylbiguanide hydrochloride and is an oral anti-diabetic drug comes under the biguanide class (Figure 1). Metformin Hydrochloride is freely soluble in water, slightly soluble in ethanol, but almost insoluble in acetone, ether, or chloroform. Metformin, marketed under the trade name Glucophage among others, is the first line medication for the treatment of type 2 diabetes, particularly in people who are overweight. It is also used in the treatment of polycystic ovary syndrome.[1] Metformin is usually described as an insulin sensitizer leading to a decline in insulin resistance and a clinically substantial reduction of plasma fasting insulin levels.[2]

Pravastatin Sodium (PS) is the sodium salt of pravastatin with cholesterol-lowering and potential antineoplastic activities (Figure 2). Pravastatin acts as a lipoprotein-lowering drug through two pathways. In the major pathway, pravastatin inhibits the function of hydroxymethylglutaryl-CoA (HMG CoA) reductase.[3] As a reversible competitive inhibitor, pravastatin sterically hinders the action of

HMG-CoA reductase by occupying the active site of the enzyme. Pravastatin also inhibits the synthesis of very-low-density lipoproteins, which are the precursor to low-density lipoproteins (LDL). These reductions increase the number of cellular LDL receptors; thus, LDL uptake increases, removing it from the bloodstream.[4]

The present study is to develop the simple, accurate and convenient method for the simultaneous estimation of Metformin Hydrochloride and Pravastatin Sodium in UV spectrophotometric method.

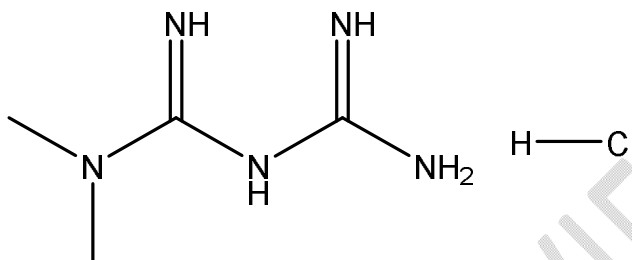


Fig. 1. Structure of metformin hydrochloride

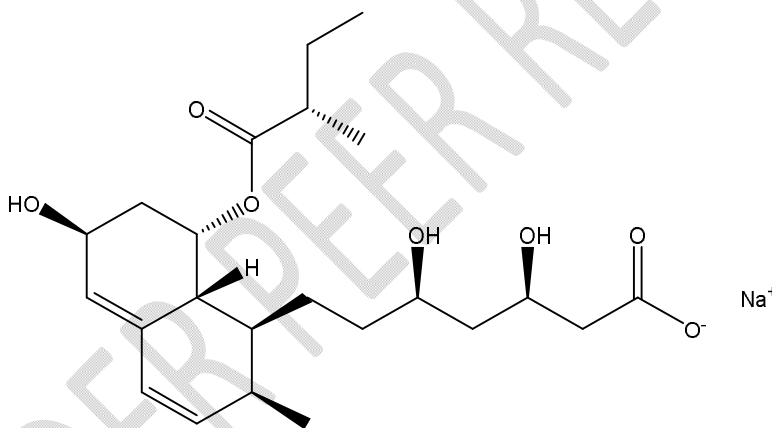


Fig. 2. Structure of Pravastatin Sodium

## 2. MATERIAL AND METHODS

All the chemicals and reagents used were of analytical grade. Metformin Hydrochloride and Pravastatin Sodium were obtained as a gift sample from Cipla Pvt. Ltd. All other chemicals were purchased from QualiChem's Lifesciences Pvt. Ltd.

### 2.1 Method Development

#### 2.1.1 UV spectrum of Metformin Hydrochloride and Pravastatin Sodium, Selection of detection wavelength

Solutions of drug were scanned over the range of 200-400nm. It was observed that both the drug showed considerable absorbance at 238nm for Pravastatin Sodium and 232nm for Metformin Hydrochloride was selected as the wavelength for preparation of standard calibration curve.

#### 2.1.2 Determination of Isoabsorptive Point

A certain Conc. of each drug was taken and scans the spectra in the range of 200-400 under the UV-Vis spectrophotometer and observes the overlay spectra of both drugs for Isoabsorptive Point.[5]

### **2.1.3 Preparation of Working Standard**

Take required quantity of 100µg/ml stock solution of Metformin Hydrochloride and Pravastatin Sodium and diluted with distilled water to obtained suitable dilutions 1-10 µg/ml for Metformin Hydrochloride and 2-20µg/ml for Pravastatin Sodium and analyzed spectrophotometrically at 238nm and 232 nm and isoabsorptive point 246nm respectively.

## **2.2 Method Validation**

### **2.2.1 Linearity**

Working standard solution of Pravastatin Sodium and Metformin Hydrochloride were taken in different 10 ml volumetric flasks and diluted up to mark with distilled water to obtained concentrations 1-10µg/ml of Metformin Hydrochloride and 2-20µg/ml of Pravastatin sodium. A calibration curve was constructed by plotting concentration versus absorbance and line equation was calculated for both the drugs.[6]

### **2.2.2 Accuracy (Recovery Study)**

Known amounts of standard solutions of Metformin Hydrochloride and Pravastatin sodium, were added at 75, 100 and 125 % level to the pre-quantified sample solutions of Metformin Hydrochloride and Pravastatin Sodium and the recovery was calculated.[7]

### **2.2.3 Repeatability**

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of the solutions (n =6) of Metformin Hydrochloride sodium (8µg/ml) and Pravastatin Sodium (4µg/ml) at same time without changing the parameters of the proposed method.[8]

### **2.2.4 Intermediate Precision (Reproducibility)**

Intra-day study was performed by analyzing, the concentration of drug for six times in the same day. Inter-day precision was performed by analyzing concentration of the drug for six days in a week.[9]

### **2.2.5 Limit of Detection and Limit of Quantification**

Limit of detection can be calculated using following equation as per ICH guidelines.

$$LOD = 3.3 \times N/S$$

Where, N is the standard deviation of the intercepts of the drug and S is the slope of the corresponding calibration curve.

Limit of quantification can be calculated using following equation as per ICH guidelines.

$$LOD = 10 \times N/S$$

Where, N is the standard deviation of the intercepts of the drug and S is the slope of the corresponding calibration curve.[5]

### **2.2.6 Robustness**

Robustness of the method Small deliberate changes in the isoabsorptive wavelength ( $\pm 5$  nm) was introduced and the effects on the results were examined.[10]

### **2.2.7 Ruggedness**

In this study, the proposed methods for determination of Metformin Hydrochloride and Pravastatin Sodium were carried by two analysts.[11]

### 2.2.8 Forced Degradation Studies

This study was performed by using different ICH prescribed (acidic, basic, oxidative, thermal and photolytic) stress conditions (Q1A (R2) International Conference on Harmonization, 2003).[12]

## 3. RESULT AND DISCUSSION

### 3.1 Determination of Absorption Maxima of Metformin Hydrochloride and Pravastatin sodium

UV absorption maximum of Metformin Hydrochloride and Pravastatin Sodium in methanol was determined and exhibited characteristic absorption at 232 and 238 nm respectively. UV spectrum scan of Metformin Hydrochloride and Pravastatin Sodium in water was demonstrated in figure 3.

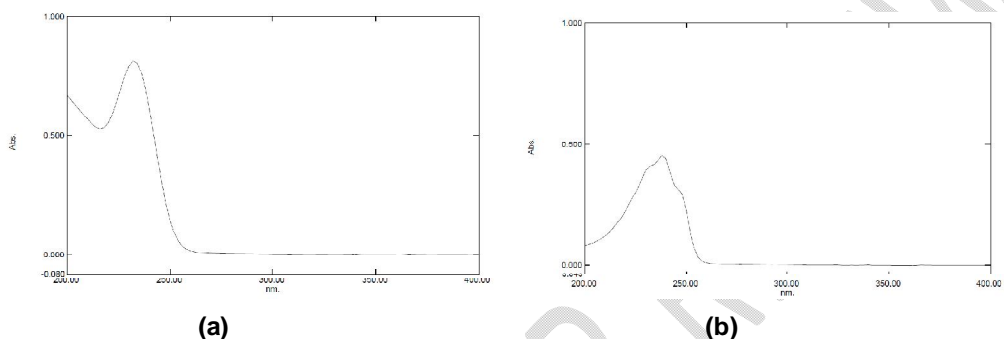


Fig. 3. UV absorption spectra of Metformin Hydrochloride and Pravastatin Sodium

### 3.2 Identification of Isoabsorptive Point

Solutions of Metformin hydrochloride(10 $\mu$ g/ml) and Pravastatin Sodium (10 $\mu$ g/ml) were scanned between 200-400 nm and the isoabsorptive point was found at 246nm (figure 4).

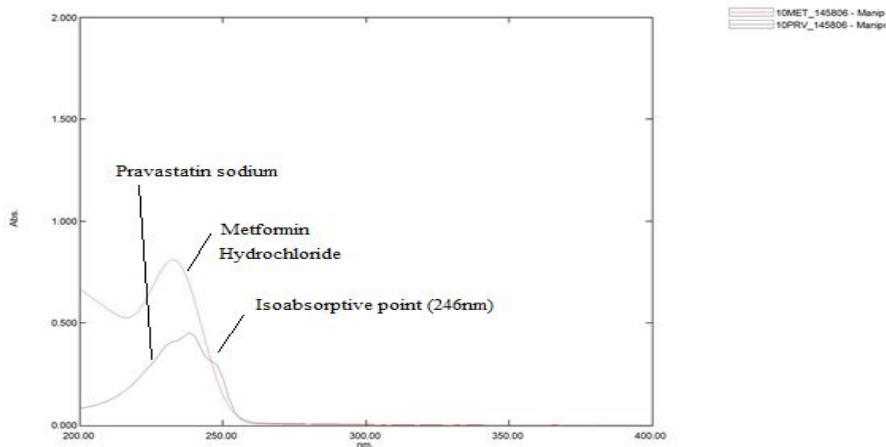


Fig. 4. Overlay spectra of Metformin Hydrochloride and Pravastatin sodium

## 3.3 Method Validation

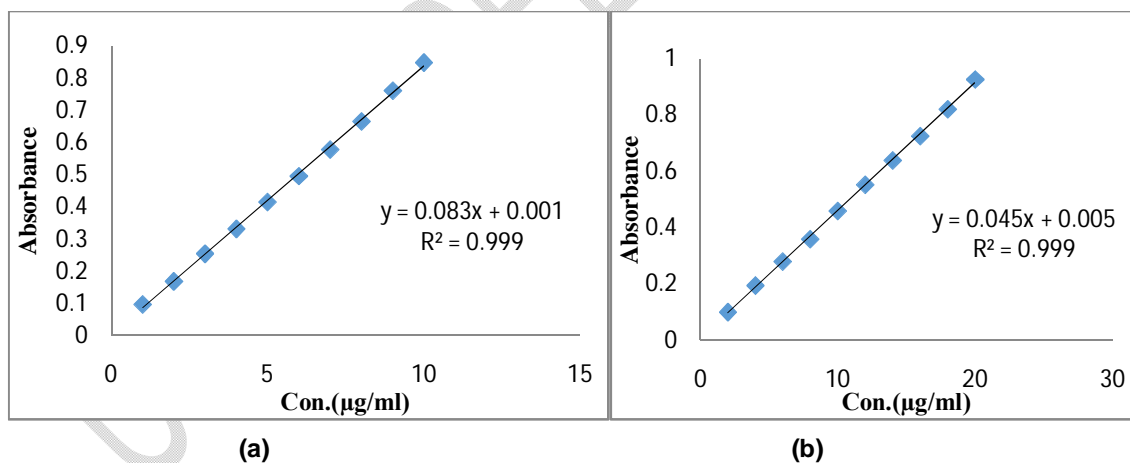
### 3.3.1 Linearity

Linearity of Metformin Hydrochloride and Pravastatin Sodium at their respective wavelength and isoabsorptive point is given in Table 1 and Figure 5.

**Table 1. Standard calibration curve of Metformin Hydrochloride in water at 232nm**

Conc. (µg/ml)	Abs. (232nm)	SD	%RSD	Conc. (µg/ml)	Abs. (238nm)	SD	%RSD
1	0.096	0.001	0.941	2	0.099	0.0004	0.404
2	0.168	0.0015	0.892	4	0.194	0.0018	0.927
3	0.254	0.0018	0.708	6	0.279	0.0017	0.609
4	0.331	0.0014	0.422	8	0.358	0.0027	0.754
5	0.415	0.0027	0.650	10	0.459	0.0039	0.849
6	0.495	0.0029	0.585	12	0.551	0.0024	0.435
7	0.578	0.0055	0.951	14	0.638	0.0018	0.282
8	0.665	0.0017	0.255	16	0.724	0.0061	0.842
9	0.761	0.0058	0.762	18	0.819	0.002	0.2442
10	0.848	0.0069	0.813	20	0.925	0.0071	0.767

\*mean±SD (N=3), Conc. = Concentration, Abs. = Absorbance, %RSD= Percent Relative Standard Deviation, SD= Standard Deviation



**Fig. 5. Standard calibration curve (a) Metformin Hydrochloride (b) Pravastatin Sodium**

Linearity and range under the experimental conditions described, the graph obtained for UV spectrum at their  $\lambda$  max at 232nm. Regression analysis was made for the slope, intercept and correlation coefficient values. The regression equations of calibration curves were  $y = 0.083x + 0.001$  ( $R^2 = 0.999$ ) at 232nm for Metformin Hydrochloride and the range was found to be 1-10 µg/ml. Linearity and range under the experimental conditions described, the graph obtained for UV spectrum at their  $\lambda$  max at 238nm. Regression analysis was made for the slope, intercept and correlation coefficient values. The

regression equations of calibration curves were  $y = 0.045x + 0.005$  ( $R^2 = 0.999$ ) at 238nm for Pravastatin Sodium and the range was found to be 2-20  $\mu\text{g/ml}$ .

### 3.3.2 Absorbance subtraction method

This method is based on the absorption factor method and its use to analysis of isosbestic point present in zero order absorption spectra known as the isoabsorptive point, where the components exhibiting this point have equal absorptivity's. The only requirements of this method (AS) are the existence of isoabsorptive point of both components and the extension of the spectra of one component.[13]

For the determination of Metformin Hydrochloride and Pravastatin sodium, we will utilize their isoabsorptive point at 246 nm. By the analysis of the recorded absorbance at the isoabsorptive point, the absorbance corresponding to Metformin Hydrochloride or Pravastatin sodium, separately, at isoabsorptive point 246 nm can be calculated using absorbance factor [abs 246 / abs 232] which is the average of the absorbance of different concentrations of pure Metformin Hydrochloride using isoabsorptive point at 246 nm to that at 232 nm which shows no contribution of Pravastatin Sodium and then the absorbance of Metformin Hydrochloride and can be obtained after subtraction.[14]

Absorbance of Metformin Hydrochloride in the mixture at  $\lambda$  246 =  $\text{abs}_{246} / \text{abs}_{232}$

(absorption factor)  $\times$  abs  $\lambda$  246 (Metformin Hydrochloride + Pravastatin sodium).

Absorbance of Pravastatin Sodium in the mixture at  $\lambda$  246 =  $\text{abs}_{\lambda 246} [( \text{Metformin Hydrochloride} + \text{Pravastatin sodium} ) - \{ \text{abs}_{246} / \text{abs}_{232} \times \text{abs}_{\lambda 232} ( \text{Metformin Hydrochloride} + \text{Pravastatin sodium} ) \}]$ .

Where, abs  $\lambda$  Metformin Hydrochloride + Pravastatin Sodium is the absorbance of the binary mixture at 232 nm and abs 246, abs232 is the absorbance factor of pure Metformin Hydrochloride at 246 nm to 232 nm and it was calculated and found to be 0.38.

The calculated absorbance value corresponding to Metformin Hydrochloride and Pravastatin Sodium can be separately used to identify each of their concentration using the unified regression equations using isoabsorptive point 246 nm.

The advantage of the absorbance subtraction method (AS) over the conventional isoabsorptive point is that there is no need for another complementary spectrophotometric method to measure the concentration of one of the two components to get the second by subtraction.[15][14]

Absorption Factor is calculated by the formula mentioned below

$$\text{Absorption factor} = \frac{\text{Absorbance at Isoabsorptive Point}}{\text{Absorbance at absorbance maxima of drug}}$$

Absorbance factor of MH and PS was as given below in Table 2 and Table 3. Absorption factor for MH and PS was found to be 0.384 and 0.685.

**Table 2. Absorbance factor of Metformin hydrochloride**

Conc.( $\mu\text{g/ml}$ )	Absorbance at 246nm	Absorbance at 232 nm	Absorption factor
1	0.038	0.096	0.395
2	0.064	0.168	0.380

3	0.094	0.254	0.370
4	0.124	0.331	0.374
5	0.158	0.415	0.380
6	0.192	0.495	0.387
7	0.23	0.578	0.397
8	0.257	0.665	0.386
9	0.298	0.761	0.391
10	0.324	0.848	0.382
Average			0.384

**Table 3. Absorbance factor of Pravastatin Sodium**

Con.(µg/ml)	Absorbance at 246nm	Absorbance at 238nm	Absorption factor
2	0.06	0.099	0.606
4	0.121	0.194	0.623
6	0.188	0.279	0.673
8	0.24	0.358	0.670
10	0.304	0.459	0.662
12	0.37	0.551	0.671
14	0.455	0.638	0.713
16	0.529	0.724	0.730
18	0.614	0.819	0.749
20	0.7	0.925	0.756
Average			0.685

### **3.3.3 Accuracy**

These studies were performed at three different levels (75%, 100% and 125%) and the % recovery of Metformin Hydrochloride (MH) and Pravastatin Sodium (PS) was calculated. The mean % recoveries were in between 98.024-98.696% and 100.593-102.378 for Metformin Hydrochloride and Pravastatin Sodium respectively as shown in Table 4.

**Table 4. Accuracy results of Metformin Hydrochloride and Pravastatin Sodium**

Con. (µg/ml)	% Recov. MH	% Recov. PS
6 (µg/ml)	97.697	102.670
6 (µg/ml)	98.089	102.891
6 (µg/ml)	98.286	101.573
Mean	98.024	102.378
Std	0.299	0.705
%RSD	0.305	0.689

8 (µg/ml)	98.450	101.528
8(µg/ml)	98.745	100.265
8 (µg/ml)	98.892	101.421
Mean	98.696	101.071
Std	0.224	0.699
%RSD	0.227	0.692
10 (µg/ml)	98.667	100.710
10 (µg/ml)	98.431	100.006
10 (µg/ml)	98.785	101.062
Mean	98.628	100.593
Std	0.179	0.537
%RSD	0.182	0.534

\*mean±SD (n=3), % Recov. = Percent Recovery, SD= Standard deviation, %RSD= Percent relative Standard deviation

### 3.3.4 Repeatability

The precision (system, method) of the proposed method was evaluated by carrying out six independent assays of test sample. RSD (%) of six assay values obtained was calculated. The intermediate precision was carried out by analyzing the sample in different days. The % RSD and % assay for repeatability and inter day precision was found to be 0.211, 0.793, 98.59%, 100.54% and 0.203, 0.780, 98.79%, 100.29% for Metformin Hydrochloride and Pravastatin Sodium respectively (Table 5 and Table 6).

**Table 5. Repeatability data of Metformin Hydrochloride and Pravastatin sodium**

Conc. (µg/ml)	% Recov. MH	Conc. (µg/ml)	% Recov.PS
8	98.598	4	101.254
	98.303		101.802
	98.4509		100.1
	98.745		100.265
	98.892		99.991
	98.598		99.82
Mean	98.598	Mean	100.54
Std	0.208	Std	0.798
%RSD	0.211	%RSD	0.793

\*mean±SD (n=6), % Recov. = Percent Recovery, SD= Standard deviation, %RSD= Percent relative Standard deviation, Conc. = Concentration.

**Table 6. Interday Precision data of Metformin Hydrochloride and Pravastatin Sodium**

Conc. (µg/ml)	Day	% Recov. MH	Conc. (µg/ml)	Day	% Recov. PS
8	1	98.450	4	1	100.814
	2	98.745		2	100.98
	3	98.892		3	99.9914
	4	99.039		4	99.002
	5	98.745		5	100.98
	6	98.892		6	99.991
	Mean	98.794		Mean	100.293
	Std	0.201		Std	0.7825
	%RSD	0.203		%RSD	0.780

\*mean±SD (n=6), % Recov. = Percent Recovery, SD= Standard deviation, %RSD= Percent relative Standard deviation, Conc. = Concentration.

### 3.3.5 Limit of Detection and Limit of Quantitation:

The LOD and LOQ were found to be 0.481µg/ml and 0.670µg/ml for Metformin Hydrochloride and 1.15µg/ml and 1.68µg/ml for Pravastatin Sodium respectively.

### 3.3.6 Ruggedness

To evaluate the ruggedness of the proposed UV method, the analysis was performed by different analysts and employing different brands of chemicals and solvents. The results presented in Table 7 indicated that the selected method was unaffected and hence rugged.

Table 7. Ruggedness data of Metformin Hydrochloride and Pravastatin Sodium

Analyst 1				Analyst 2			
Conc (µg/ml)	% Recov. MH	Conc (µg/ml)	% Recov. PS		% Recov. MH	Conc (µg/ml)	% Recov. PS
8	98.450	4	100.814	8	99.039	4	99.717
	98.745		100.265		98.745		100.98
	98.892		101.42		98.892		100.705
	98.745		100.98		99.039		99.717
	98.892		99.991		99.039		101.145
	98.745		101.694		98.892		99.991
Mean	98.745	Mean	100.860	Mean	98.941	Mean	100.376
Std	0.161	Std	0.653	Std	0.120	Std	0.645
%RSD	0.163	%RSD	0.647	%RSD	0.121	%RSD	0.642

\*mean±SD (n=6), % Recov. = Percent Recovery, SD= Standard deviation, %RSD= Percent relative Standard deviation, Conc. = Concentration.

### 3.3.7 Robustness

To evaluate the robustness of the method, the optimized method parameters were varied at different levels. The results presented in Table 8 indicated that the developed method was unaffected by small variations in the optimized method parameters.

**Table 8. Robustness data of Metformin Hydrochloride and Pravastatin Sodium**

Wavelength (nm)	Conc (µg/ml)	% Recov. MH	Conc (µg/ml)	% Recov. PS
241	8	97.861	4	96.197
241	8	97.714	4	95.75
241	8	98.156	4	96.362
	Mean	97.911	Mean	96.10
	Std	0.224	Std	0.313
	%RSD	0.229	%RSD	0.325
246	8	98.450	4	101.528
246	8	98.745	4	100.265
246	8	98.892	4	101.42
	Mean	98.696	Mean	101.071
	Std	0.224	Std	0.699
	%RSD	0.227	%RSD	0.692
251	8	97.861	4	98.34
251	8	98.156	4	98.505
251	8	98.009	4	97.351
	Mean	98.009	Mean	98.065
	Std	0.147	Std	0.6241
	%RSD	0.1502	%RSD	0.636

\*mean±SD (n=3), % Recov. = Percent Recovery, SD= Standard deviation, %RSD= Percent relative Standard deviation, Conc. = Concentration.

### 3.3.8 Forced Degradation study

Forced degradation studies were performed to demonstrate the stability of the sample. Degradation studies were carried out under conditions of hydrolysis, oxidation, UV light, forced degradation profile of Metformin Hydrochloride and Pravastatin Sodium is as given below in Table 9 to Table 12.

**Table 9. Degradation profile of Metformin Hydrochloride and Pravastatin Sodium in acidic media**

Conc (µg/ml)	% Recov. MH	% Recov. MH	Conc (µg/ml)	% Recov. PS	% Recov. PS
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8	90.941	9.058	4	94.088	5.911
8	91.088	8.911	4	94.528	5.471
8	90.5	9.5	4	95.625	4.374
Mean	90.843	9.156	Mean	94.747	5.252
STD	0.306	0.306	STD	0.7916	0.791

\*mean±SD (n=3), % Recov. = Percent Recovery, SD= Standard deviation, Conc. = Concentration.

**Table 10. Degradation profile of Metformin Hydrochloride and Pravastatin Sodium in basic media**

Conc (µg/ml)	% Recov. Met	% Recov. Met	Conc (µg/ml)	% Recov. PS	% Recov. PS
8	94.180	5.819	4	95.197	4.802
8	94.328	5.671	4	95.637	4.362
8	94.475	5.524	4	93.934	6.065
Mean	94.328	5.671	Mean	94.922	5.077
STD	0.147	0.147	STD	0.883	0.883

\*mean±SD (n=3), % Recov. = Percent Recovery, SD= Standard deviation, Conc. = Concentration.

**Table 11. Degradation profile of Metformin Hydrochloride and Pravastatin Sodium in oxidizing media**

Conc (µg/ml)	% Recov. MH	% Recov. MH	Conc (µg/ml)	% Recov. PS	% Recov. PS
8	86.819	13.180	4	83.197	16.802
8	86.966	13.033	4	83.637	16.362
8	86.671	13.328	4	84.9	15.1
Mean	86.819	13.180	Mean	83.911	16.088
STD	0.147	0.147	STD	0.883	0.883

\*mean±SD (n=3), % Recov. = Percent Recovery, SD= Standard deviation, Conc. = Concentration.

**Table 12. Degradation profile of Metformin Hydrochloride and Pravastatin Sodium under UV light**

Conc (µg/ml)	% Recov. MH	% Recov. MH	Conc (µg/ml)	% Recov. PS	% Recov. PS
8	92.708	7.291	4	93.654	6.345
8	92.414	7.585	4	93.488	6.511
8	93.003	6.996	4	93.82	6.18
Mean	92.708	7.291	Mean	93.654	6.345
STD	0.294	0.294	STD	0.165	0.1657

\*mean±SD (n=3), % Recov. = Percent Recovery, SD= Standard deviation, Conc. = Concentration.

Acid hydrolysis was performed by treating the drug with 2N HCl and percentage degradation of both Metformin Hydrochloride and Pravastatin Sodium was found to be  $9.15 \pm 0.30$  and  $5.25 \pm 0.79$ . In basic media percentage degradation was found to be  $5.67 \pm 0.14$ ,  $5.07 \pm 0.88$ , in oxidizing media  $13.18 \pm 0.14$ ,  $16.08 \pm 0.88$ , Under UV light degradation was  $7.29 \pm 0.29$ ,  $6.34 \pm 0.16$ .

#### 4. CONCLUSION

Absorption–subtraction methods was developed for the estimation of Metformin Hydrochloride and Pravastatin Sodium in their combined dosage form. On UV spectrophotometer analysis absorption maxima of MH and PS was found to be 232 and 238nm respectively, and the isoabsorptive point of each drug was found at 246 nm. These studies were performed at three different levels (75%, 100% and 125%) and the % recovery of MH and PS was calculated. The LOD and LOQ were found to be  $0.481 \mu\text{g/ml}$  and  $0.670 \mu\text{g/ml}$  for Metformin Hydrochloride and  $1.15 \mu\text{g/ml}$  and  $1.68 \mu\text{g/ml}$  for Pravastatin Sodium respectively. All the statically analyses were within the standard limits. It proves that the method was repeatable and selective for the simultaneous. Therefore, the present study concludes that it can be successfully used for simultaneous estimation of Metformin Hydrochloride and Pravastatin Sodium in pharmaceutical dosage forms. The developed method was found to be simple, precise, and accurate.

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