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**FIRST ORDER DERIVATIVE AND DUAL WAVELENGTH SPECTROPHOTOMETRY METHODS DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF AVANAFIL AND DAPOXENTINE HYDROCHLORIDE IN BULK AND DOSAGE FORM**

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**Abstract**

To develop and validate a simple, economical, sensitive and accurate Spectrophotometry methods for simultaneous estimation of Avanafil and Dapoxetine hydrochloride in their combined pharmaceutical dosage form. Two simple, accurate, precise, reproducible and economical U.V Spectroscopy methods have been developed. First method was based on First order derivative technique. Here 246.60 nm, the zero crossing point of Avanafil, was selected for the estimation of Dapoxetine hydrochloride and 210 nm, the zero crossing point of Dapoxetine hydrochloride, was selected for the estimation of Avanafil. The second method was the dual wavelength method, where 206.80 nm and 214.80 nm were selected as  $\lambda_1$  and  $\lambda_2$  for the estimation of Avanafil and 241.20 nm and 251.60 nm were selected for the estimation of Dapoxetine hydrochloride. Avanafil and Dapoxetine hydrochloride showed linearity in the range of 1-8 $\mu$ g/ml and 1-8 $\mu$ g/ml respectively in derivative and 1-6 $\mu$ g/ml and 1-3.5 $\mu$ g/ml in dual wavelength methods of Spectrophotometry. Correlation coefficient for all methods was greater than 0.999. Both methods were validated by validation parameters and it show result where lie within its acceptance criteria as per ICH Q2 (R1) guideline. Hence, it can be successfully used for the routine analysis of Avanafil and Dapoxetine hydrochloride in their combined pharmaceutical dosage forms.

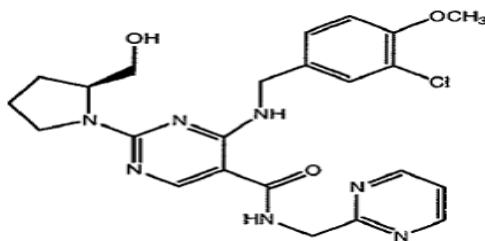
**Key word:** Avanafil, Dapoxetine HCL, Validation, ICH.

**Introduction**

Super Avana is a medication for the treatment of erectile dysfunction, is a combination of Avana is combination of Avanafil and Dapoxetine HCL has been approved by the FDA. Avanafil is a selective inhibitor of cGMP-specific PDE5.

Avanafil is designated chemically as (S)-4-[(3-Chloro-4 methoxybenzyl) amino]-2-[2-(hydroxymethyl)-1-pyrrolidiny]-N-(2-pyrimidinylmethyl)-5 pyrimidinecarboxamide. Avanafil occurs as white crystalline powder. Its Molecular formula and Molecular weight are  $C_{23}H_{26}ClN_7O_3$  & 483.95 respectively.

The structural formula is:

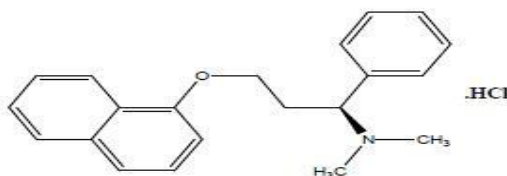


**Figure 1: chemical structure of Avanafil.**

It is slightly soluble in ethanol, practically insoluble in water, soluble in 0.1 mol/L hydrochloric acid.

Dapoxetine HCL. It is Selective serotonin reuptake inhibitors. Its chemical name is (S)-N, N dimethyl- 3-(naphthalen-1-yloxy)-1-phenylpropan-1-amine hydrochloride. Its Molecular formula and Molecular weight are  $C_{21}H_{24}ClNO$  & 341.88

The structural formula is:



**Figure 2: chemical structure of Dapoxetine HCL.**

Its solubility is water – 100 mg/ML, 0.01 N Hcl – 71 mg/ML, Citrate PH 2 – 0.9 mg/ML phosphate pH 7 – 0.02mg/ML.

The mechanism of action of Dapoxetine in premature ejaculation is presumed to be linked to the inhibition of neuronal reuptake of serotonin and the subsequent potentiation of the neurotransmitter's action at pre and post synaptic receptor.

It can cause side effect like Dizziness, Nausea, Anxiety, Restlessness, Depression, Mood change, Blurred vision,

Vertigo, Vomiting.<sup>1</sup>Up to now there are methods developed on Avanafil and Dapoxetine HCL. <sup>2-14</sup> but there are no

Spectrophotometry methods developed on Simultaneous estimation of Avanafil and Dapoxetine HCL.

## Martials and Methods

### Instrumentation, Reagents and Material

Jasco UV-1800 UV spectrophotometer, Avanafil, Dapoxetine HCL, 0.1N HCL

## Marketed formulation

The commercial formulation Super Avana which each contains 100mg Avanafil and 60mg Dapoxetine HCL.

## Preparation of standard solution

### *Preparation of standard stock solution of Avanafil*

Accurately weighed quantity of Avanafil 100 mg was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with 0.1N HCL. This will give a stock solution having strength of 1000 µg/ml.

### *Preparation of working standard solution of Avanafil*

100 µg/ml of Avanafil solution was prepared by diluting 10 ml of stock solution to 100 ml with 0.1N HCL

### *Preparation of standard stock solution of Dapoxetine HCL*

Accurately weighed quantity of Dapoxetine HCL 100 mg was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with 0.1N HCL. This will give a stock solution having strength of 1000 µg/ml.

### *Preparation of working standard solution of Dapoxetine HCL*

100 µg/ml of Dapoxetine HCL solution was prepared by diluting 10 ml of stock solution to 100 ml with 0.1N HCL.

## First Order Derivative Method

### Derivative Conditions:

Mode:Spectrum, Scan speed:Medium, Wavelength range:200-400 nm, Initial base line correction:0.1N HCL, Derivative order:1, the first order derivative spectra of each solution were obtained using smoothing  $\Delta\lambda = 8$  and scaling factor =25.

### 2.4.2 Determination of wavelength for measurement

0.5 ml of working standard solution of Avanafil (100 µg/ml) and 0.2 ml of working standard of Dapoxetine HCL (100 µg/ml) was diluted to 10 ml with 0.1N HCL to get 5 µg/ml of Avanafil and 2 µg/ml of Dapoxetine HCL. Each solution was scanned between 200-400 nm. The first order derivative spectra of each solution were obtained. ZCP of Avanafil was found 246.60 nm and ZCP of Dapoxetine HCL was found 210 nm. The zero crossing point (ZCP) of Avanafil at which Dapoxetine HCL is measured and ZCP of Dapoxetine HCL at which Avanafil is measured, obtained from the overlain spectra of both. Which shown in figure no. 1.1.

### 2.4.3 Preparation of Calibration Curve:

#### *Calibration curve for Avanafil (1-7 µg/ml)*

Calibration curve for Avanafil consisted of different concentrations of standard Avanafil solution ranging from 1-6 $\mu$ g/ml. The solutions were prepared by pipetting out 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7ml of the working standard solution of Avanafil (100  $\mu$ g/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with 0.1N HCL. The first derivative (D1) curve of each solution against the 0.1N HCL was recorded. D1 absorbance at ZCP of Dapoxetine HCL was measured and the plot of D1 absorbance vs. concentration was plotted. The straight-line equation was determined. And data was recorded in table no. 1.1 and figure no. 1.2- 1.3.

*Calibration curve for Dapoxetine HCL(1-7 $\mu$ g/ml):* Calibration curve for Dapoxetine HCL consisted of different concentrations of standard Dapoxetine HCL solution ranging from 1-7 $\mu$ g/ml. The solutions were prepared by pipetting 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7ml of the working standard solution of Dapoxetine HCL (100  $\mu$ g/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with 0.1N HCL. The first derivative (D1) curve of each solution against the 0.1N HCL was recorded. D1 absorbance at ZCP of Avanafil was measured and the plot of D1 absorbance vs. concentration was plotted. The straight-line equation was determined. And data was recorded in table no. 1.1 and figure no. 1.2- 1.4.

#### 2.4.4 Validation of proposed method

**Linearity:** The linearity response was determined by analyzing independent levels of concentrations in the range of 1-7 and 1-7 $\mu$ g/ml for Avanafil and Dapoxetine HCL respectively six times. Absorbance of each solution was measured at ZCP of Dapoxetine HCL and Avanafil respectively using developed method. Calibration curve of D1 absorbance vs. concentration was plotted. The correlation coefficient and regression line equations for Avanafil and Dapoxetine HCL were determined. Linearity of 6 concentrations were measured six times and recorded in table no. 1.2.

**Precision:**

**Repeatability:** 6 replicates of 5 $\mu$ g/ml concentrations of Avanafil and 3 $\mu$ g/ml of Dapoxetine HCL were prepared and absorbance was measured at ZCP of Dapoxetine HCL and Avanafil respectively. SD and RSD were calculated and recorded in table no. 1.3.

**Intraday Precision:** Standard solutions containing 4, 5 and 6 $\mu$ g/ml Avanafil and 2, 3 and 4 $\mu$ g/ml Dapoxetine HCL were analyzed 3 times on the same day. The absorbance of solutions was measured at ZCP of Dapoxetine HCL and Avanafil respectively. SD and RSD were calculated and recorded in table no. 1.4.

**Interday Precision:** Standard solutions containing 4, 5 and 6µg/ml Avanafil and 2, 3 and 4µg/ml µg/ml Dapoxetine HCL were analyzed 3 times on the three different days. The absorbance of solutions was measured at ZCP of Dapoxetine HCL and Avanafil respectively. SD and RSD were calculated and recorded in table no. 1.5.

### Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. Recovery studies were carried out by addition of standard drug to the pre analysed sample at 3 different concentration levels (80, 100 and 120 %) taking into consideration percentage purity of added bulk drug samples. It was determined by calculating the recovery of Avanafil and Dapoxetine HCL Sodium by standard addition method.

#### *Preparation of sample solution for % recovery:*

An accurately weighed powder equivalent to about 100mg Avanafil was transferred to 100 ml volumetric flask and the volume was made up to the mark using 0.1N HCL as solvent and aliquate them to make final concentration 5µg/ml Avanafil and 3 µg/ml Dapoxetine HCL. The resulting solution was filtered through Whatman filter paper. Absorbance of sample solutions was measured at selected wavelength of Avanafil and Dapoxetine HCL and concentration is calculated which is known as pre-analyzed sample.

In pre-analyzed sample 80, 100 and 120 % of Avanafil and Dapoxetine HCL was spiked. Absorbance of spiked samples was measured and total amount of drug was calculated and from which % recovery was calculated and recorded in table no. 1.6 & 1.7.

### Limit of Detection (LOD)

The LOD is estimated from the set of 6 calibration curves used to determine method linearity. The LOD may be calculated as;

$$\text{LOD} = 3.3 \times (\text{SD} / \text{Slope})$$

Where, SD = the standard deviation of Y- intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

### Limit of Quantification (LOQ)

The LOQ is estimated from the set of 6 calibration curves used to determine method linearity. The LOQ may be calculated as;

$$\text{LOQ} = 10 \times (\text{SD} / \text{Slope})$$

Where, SD = the standard deviation of Y- intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

Which are shown in table no 1.8

### **Analysis of marketed formulation:**

Twenty tablets were weighed and content crushed to obtain a fine powder. An accurately weighed powder equivalent to about 10 mg of Avanafil was transferred to 100 ml volumetric flask and the volume was made up to the mark using 0.1N HCL as solvent. The solution was sonicated for 20 minutes. The solution was filtered through Whatman Filter Paper No.42. First 0.5 ml of filtrate were discarded and was diluted to 10 ml with 0.1N HCL. Resulting solution contains 5 µg/ml Avanafil and 3 µg/ml Dapoxetine HCL. The absorbance of the resulting solution was measured at 210 nm for Avanafil and 246.60 nm for Dapoxetine HCL. The concentration of each drug was calculated using equation of straight line. This is shown in figure no. 1.5 and table no. 1.9.

### **Development of Dual Wavelength Method**

#### **Determination of wavelength for measurement**

0.5 ml of working standard solution of Avanafil (100 µg/ml) and 0.2 ml of working standard of Dapoxetine HCL (100 µg/ml) was diluted to 10 ml with 0.1N HCL to get 5 µg/ml of Avanafil and 2 µg/ml of Dapoxetine HCL. Each solution was scanned between 200-400 nm. From the overlay spectra two wavelengths 214.80 nm and 206.80 nm were selected as  $\lambda_1$  and  $\lambda_2$  for the estimation of Avanafil. Dapoxetine HCL shows the same absorbance at these wavelengths. Similarly, wavelengths 241.20 nm and 251.60 nm were selected as  $\lambda_3$  and  $\lambda_4$  for estimation of Dapoxetine HCL. Avanafil shows the same absorbance at these wavelengths which is shown in figure no. 2.1.

#### **Preparation of Calibration Curve:**

##### *Calibration curve for Avanafil (1-6 µg/ml)*

Calibration curve for Avanafil consisted of different concentrations of standard Avanafil solution ranging from 1-6 µg/ml. The solutions were prepared by pipetting out 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 ml of the working standard solution of Avanafil (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with 0.1N HCL. The absorbance of the solutions was measured at 214.80 nm and 206.80 nm against 0.1N HCL and the plot of absorbance

differences vs. concentration was plotted. The straight-line equation was determined and shown in table no 2.1 and figure no. 2.2 and 2.4.

#### *Calibration curve for Dapoxetine HCL(1-3.5µg/ml)*

Calibration curve for Dapoxetine HCL consisted of different concentrations of standard Dapoxetine HCL solution ranging from 1-3.5 µg/ml. The solutions were prepared by pipetting out 0.1, 0.15, 0.2, 0.25, 0.3, and 0.35ml of the working standard solution of Dapoxetine HCL (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with 0.1N HCL. The absorbances of the solutions were measured at 241.20 nm and 251.60 nm against 0.1N HCL and the plot of absorbance differences vs. concentration was plotted. The straight-line equation was determined and shown in 2.1 and figure no. 2.3 and 2.5.

### **2.5.3 Validation of proposed method**

#### **Linearity**

The linearity response was determined by analyzing independent levels of concentrations in the range of 1-6 and 1-3.5µg/ml for Avanafil and Dapoxetine HCL respectively 6 times.. Absorbance of each solution was measured at selected wavelength respectively using developed method. Calibration curve of absorbance differences vs. concentration was plotted. The correlation coefficient and regression line equations for Avanafil and Dapoxetine HCL were determined. Linearity of 6 concentrations were measured six times and recorded in table no. 2.2.

#### **Precision**

**Repeatability:** 6 replicates of 2 µg/ml concentrations of Avanafil and 1.5 µg/ml of Dapoxetine HCL were prepared and absorbance was measured at selected wavelength respectively. SD and RSD were calculated and recorded in table no. 2.3.

**Intraday Precision:** Standard solutions containing 3, 4 and 5 µg/ml Avanafil and 2, 2.5 and 3.5 µg/ml Dapoxetine HCL were analyzed 3 times on the same day. The absorbance of solutions was measured at selected wavelength respectively. SD and RSD were calculated and recorded in table no. 2.4.

**Interday Precision:** Standard solutions containing 3, 4 and 5 µg/ml Avanafil and 2, 2.5 and 3.5 µg/ml Dapoxetine HCL were analyzed on 3 different days. The absorbance of solutions was measured at selected wavelength respectively. SD and RSD were calculated and recorded in table no. 2.5

## Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. Recovery studies were carried out by addition of standard drug to the pre analysed sample at 3 different concentration levels (80, 100 and 120 %) taking into consideration percentage purity of added bulk drug samples. It was determined by calculating the recovery of Avanafil and Dapoxetine HCL by standard addition method.

### *Preparation of sample solution for % recovery:*

An accurately weighed powder equivalent to about 100 mg of Avanafil was transferred to 100 ml volumetric flask and the volume was made up to the mark using 0.1N HCL as solvent and aliquate them to make final concentration 5 µg/ml Avanafil and 3 µg/ml Dapoxetine HCL. The resulting solution was filtered through Whatman filter paper. Absorbance of sample solutions was measured at selected wavelength of Avanafil and Dapoxetine HCL and concentration is calculated which is known as pre-analyzed sample.

In pre-analyzed sample 80, 100 and 120 % of Avanafil and Dapoxetine HCL was spiked. Absorbance of spiked samples was measured and total amount of drug was calculated and from which % recovery was calculated and recorded in table no. 2.6 & 2.7.

## Limit of Detection (LOD)

The LOD is estimated from the set of 6 calibration curves used to determine method linearity. The LOD may be calculated as;

$$\text{LOD} = 3.3 \times (\text{SD} / \text{Slope})$$

Where, SD = the standard deviation of Y- intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

## Limit of Quantification (LOQ)

The LOQ is estimated from the set of 6 calibration curves used to determine method linearity. The LOQ may be calculated as;

$$\text{LOQ} = 10 \times (\text{SD} / \text{Slope})$$

Where, SD = the standard deviation of Y- intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.



Which are shown in table no 2.8

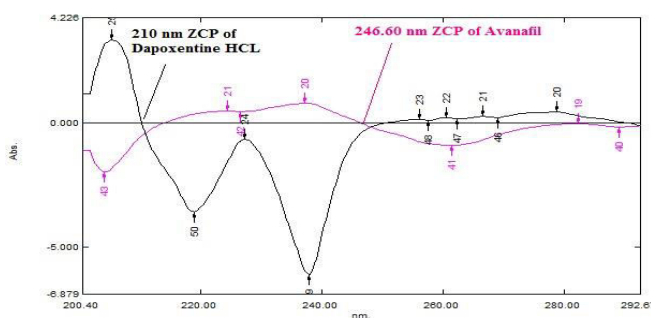
**Analysis of marketed formulation:** Twenty tablets were weighed and content crushed to obtain a fine powder. An accurately weighed powder equivalent to about 10 mg of Avanafil was transferred to 100 ml volumetric flask and the volume was made up to the mark using 0.1N HCL as solvent. The solution was sonicated for 20minutes. The solution was filtered through whatman Filter Paper No.42. First 0.5 ml of filtrate were discarded and was diluted to 10 ml with 0.1N HCL. Resulting solution contain 5µg/mlAvanafil and 3µg/mlDapoxetine HCL. The absorbance of the resulting solution was measured at 214.80 nm &206.80 nmfor Avanafil and 241.20 nm &251.60 nmfor Dapoxetine HCL. The concentration of each drug was calculated using equation of straight line.This is shown in figure no. 2.6 and table no. 2.9.

## Result and Discussion

### First Order Derivative Method

#### Selection of wavelength for simultaneous estimation of Avanafil and Dapoxetine HCL

0.5 ml of working standard solution of Avanafil (100µg/ml) and 0.2 ml of working standard solution of Dapoxetine HCL (100µg/ml) was pipette out into two separate 10 ml volumetric flask and volume was adjusted to the mark with 0.1N HCL to get 5µg/ml of Avanafil and 2µg/ml of Dapoxetine HCL. Each solution was scanned between 200-400 nm against 0.1N HCL as a reagent blank for zero order spectra. The first order derivative spectra of each solution were obtained using smoothing ( $\Delta\lambda = 8$ , Scaling Factor = 25). The zero crossing points were selected to be 246.60 nm and 210 nm for Avanafil and Dapoxetine HCL respectively. Wavelengths selected for quantitation were 246.60nm for Dapoxetine HCL (Zero crossing point for Avanafil) and 210 nm for Avanafil (zero crossing point for Dapoxetine HCL)



**Figure no. 3: First order UV spectra of Avanafil and Dapoxetine HCL showing selection of wavelength for detection.**

Standard curve:

Avanafil at 210 nm		Dapoxetine HCL at 246.60 nm	
Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
1	-0.128	1	-0.030
2	-0.230	2	-0.076
3	-0.316	3	-0.113
4	-0.431	4	-0.154
5	-0.522	5	-0.193
6	-0.621	6	-0.228
7	-0.710	7	-0.269
8	-0.814	8	-0.303

Table no. 1: Standard curve data for Avanafil and Dapoxetine HCL.

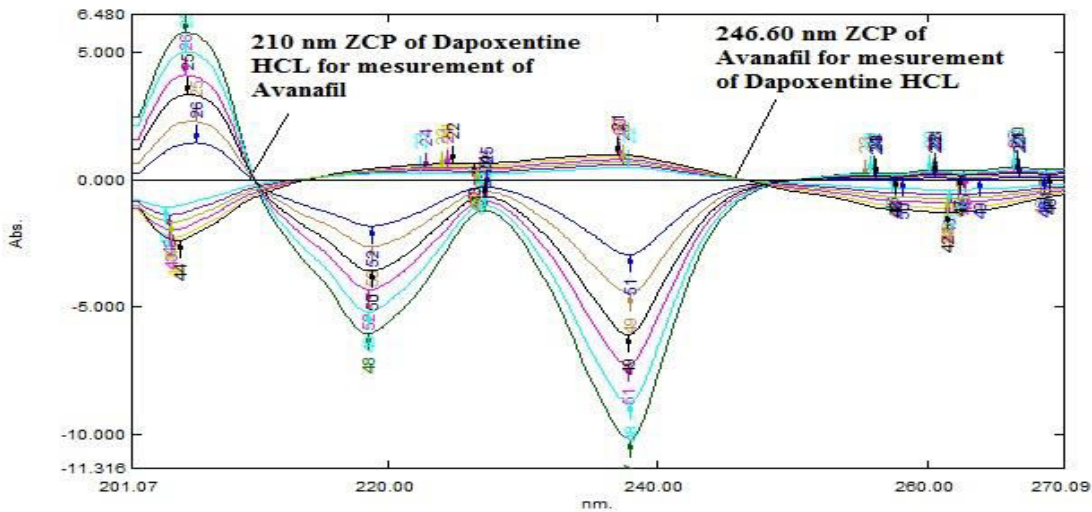


Figure no. 4: Standard curve Spectra of Avanafil and Dapoxetine HCL showing selection of wavelength for detection.

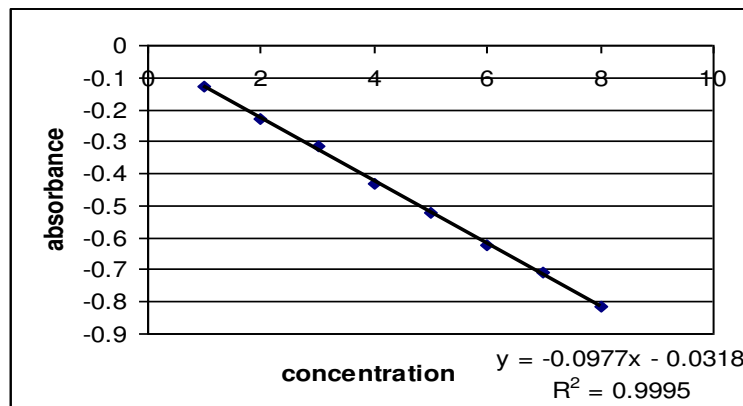


Figure no.5: STD cure for Avanafil at ZCP of Dapoxetine HCL 246.60 nm.

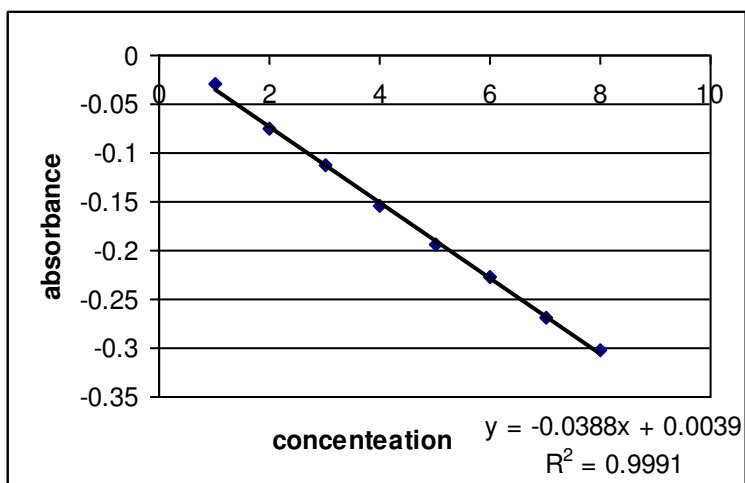


Figure no. 6: STD cure for Dapoxetine HCL at ZCP of Avanafil246.60 nm

**Conclusion:**The linearity range for Avanafil was found to be in the range of 1-6µg/ml and for Dapoxetine HCL1-7µg/ml. Correlation co-efficient for calibration curve of Avanafil and Dapoxetine HCL was found to be 0.9995 and0.9991 respectively.

Parameter	Avanafil at 246.60 nm	Dapoxetine HCL at 246.60 nm
Regression lineEquation	Y= -0.0977x -0.0318	Y= -0.0388x + 0.0039
Correlation coefficient	0.9995	0.9991

Table no. 2: STD curve result.

**Method Validation**

**Linearity**

Avanafil at 210 nm		Dapoxetine HCL at 246.60 nm	
Concentration (µg/ml)	D <sup>1</sup> Absorbance Mean* ± S.D.	Concentration (µg/ml)	D <sup>1</sup> Absorbance Mean* ± S.D.
1	-0.127±0.0010	1	-0.030±0.0005
2	-0.230±0.0014	2	-0.076±0.0001
3	-0.361±0.0018	3	-0.114±0.0018
4	-0.431±0.0024	4	-0.152±0.0014
5	-0.521±0.0024	5	-0.191±0.0018
6	-0.621±0.0023	6	-0.228±0.0018
7	-0.713±0.0030	7	-0.267±0.0021

\*n=6

Table no 3: Linearity data for Avanafil and Dapoxetine HCL.

**Precision**

**Repeatability**

Avanafil at 210 nm		Dapoxetine HCL at 246.60 nm	
Concentration (µg/ml)	D <sup>1</sup> Absorbance	Concentration (µg/ml)	D <sup>1</sup> Absorbance
5	-0.524	3	-0.116
5	-0.527	3	-0.115
5	-0.521	3	-0.113
5	-0.519	3	-0.116
5	-0.522	3	-0.114
5	-0.520	3	-0.115
<b>Mean</b>	-0.522	<b>Mean</b>	-0.114
<b>SD</b>	±0.0029	<b>SD</b>	±0.0011
<b>%RSD</b>	0.56	<b>%RSD</b>	1.01

**Table no. 4: Repeatability data for Avanafil at 210 nm and Dapoxetine HCL at 246.60 nm.**

**Discussion:** The % RSD for Repeatability of both the drugs was found to be less than 2. So, it was concluded that proposed method for estimation of Avanafil and Dapoxetine HCL is précised in nature.

**Intraday precision**

Avanafil Concentration (µg/ml)	D <sup>1</sup> Absorbance* ±S.D.	%RSD	Dapoxetine HCL Concentration (µg/ml)	D <sup>1</sup> Absorbance* ±S.D.	%RSD
4	-0.430±0.0015	0.35	2	-0.075±0.0005	0.76
5	-0.523±0.0020	0.38	3	-0.115±0.0010	0.86
6	-0.624±0.0025	0.40	4	-0.155±0.0011	0.64

\*n=3

**Table no. 5: Intraday precision data for estimation of Avanafil and Dapoxetine HCL.**

**Discussion:** The % RSD for Repeatability of both the drugs was found to be less than 2.0, so, it was concluded that proposed method for estimation of Avanafil and Dapoxetine HCL is précised in nature

**Interday precision**

Avanafil Concentration (µg/ml)	Absorbance* ±S.D.	%RSD	Dapoxetine HCL Concentration (µg/ml)	Absorbance* ±S.D.	%RSD
4	-0.423±0.0020	0.49	2	- 0.075±0.0010	1.33
5	-0.523±0.0032	0.61	3	- 0.114±0.0012	0.87
6	-0.622±0.0032	0.51	4	- 0.154±0.0020	1.29

\*n=3

**Table no. 6: Interday precision data for estimation of Avanafil and Dapoxetine HCL.**

**Discussion:** The % RSD for Repeatability of both the drugs was found to be less than 2 so, it was concluded that proposed method for estimation of Avanafil and Dapoxetine HCL is précised in nature

**Accuracy**

Level of recovery	Sample amount (µg/ml)	amount added (µg/ml)	amount recovered (µg/ml)	% recovery	AVG	SD	%RSD
80%	5	4	4.04	100.13	100.28	1.143	1.13
80%	5	4	4.06	101.5			
80%	5	4	3.96	99.23			
100%	5	5	4.97	99.4	99.81	0.425	0.42
100%	5	5	5.01	100.25			
100%	5	5	4.99	99.8			
120%	5	6	5.97	99.6	99.65	0.721	0.72
120%	5	6	5.93	98.96			
120%	5	6	6.02	100.4			

**Table no. 7: Accuracy (%Recovery) data for Avanafil.**

**Discussion:** Result obtained reveals that % recovery of Avanafil was within acceptance criteria given in ICH guideline.

Level of recovery	Sample amount (µg/ml)	amount added (µg/ml)	amount recovered (µg/ml)	% recovery	AVG	SD	%RSD
80%	3	2.4	2.42	100.62	99.29	1.610	1.62
80%	3	2.4	2.40	99.75			
80%	3	2.4	2.41	97.5			
100%	3	3	3	101	99.96	0.896	0.89
100%	3	3	3.2	99.5			
100%	3	3	3	99.4			
120%	3	3.6	3.5	99.16	99.21	0.916	0.92
120%	3	3.6	3.6	100.16			
120%	3	3.6	3.7	98.33			

**Table no. 8: Accuracy (%Recovery) data for Dapoxetine HCL.**

**Discussion:** Result obtained reveals that % recovery of Dapoxetine HCL was within acceptance criteria given in ICH guideline.

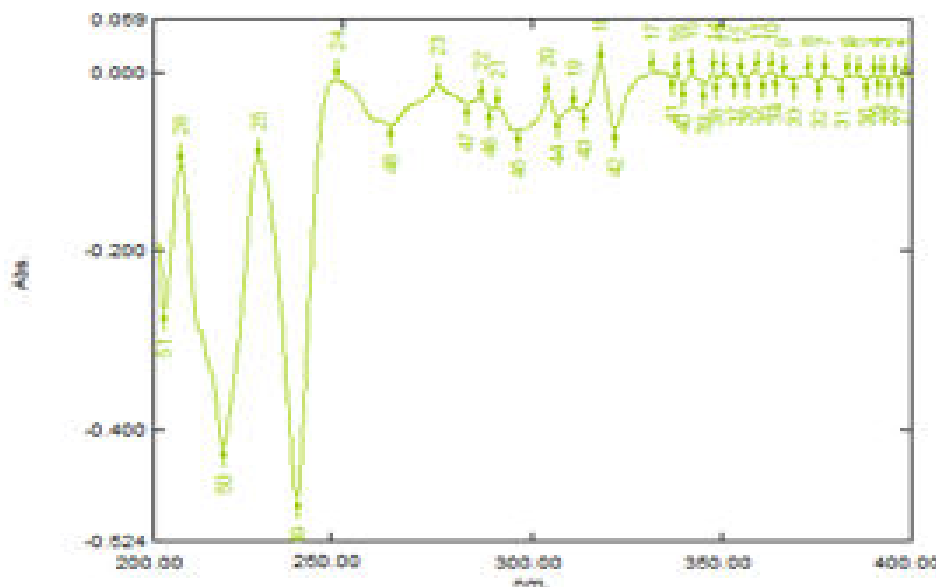
**Limit of Detection and Limit of Quantitation.**

Parameters	Avanafil	Dapoxetine HCL
Mean Slope (n=6)	-0.0313	-0.0036
SD (n=6)	±0.0003	±0.0001
LOD (µg/ml)	0.03	0.09
LOQ (µg/ml)	0.12	0.20

**Table no. 9: LOD and LOQ data for Avanafil and Dapoxetine HCL.**

**Discussion:** The proposed method can detect and quantify small amount of drugs with precisely. So, it was concluded that the proposed method is very sensitive in nature.

**Analysis of marketed formulation**



**Figure no. 7: First orders Derivative Spectrum of Marketed formulation.**

BRAND NAME: OSENI	Drugs	Label Claim (mg)	Amount Found (mg)	% Label Claim*
	Avanafil	100	99.2	99.2%
	Dapoxetine HCL	60	61.63	102%

**Table no. 10: Analysis of marketed formulation.**

**Discussion:** % Assay of Avanafil and Dapoxetine HCL was found in an acceptance limit so this method could be used for analysis of this combination.

**Dual Wavelength Method**

**Selection of wavelength for simultaneous estimation of Avanafil and Dapoxetine HCL**

By appropriate dilutions from the working standardsolutions of 100 µg/ml of Avanafil and 100 µg/ml of Dapoxetine HCL, thesolutions of Avanafil (10 µg/ml) and Dapoxetine HCL (10 µg/ml) wereprepared respectively and scanned over the range of 200- 400 nm and the overlain spectra were observed fordevelopment of suitable method for analysis.

From the overlayspectra two wavelengths 214.80 nm and 206.80 nm wereselected as  $\lambda_1$  and  $\lambda_2$ for the estimation of Avanafil. Dapoxetine HCL shows thesame absorbance at these wavelengths. Similarly,wavelengths 241.20 nm and

251.60 nm were selected as  $\lambda_3$  and  $\lambda_4$  for estimation of Dapoxetine HCL. Avanafil shows the same absorbance at these wavelengths.

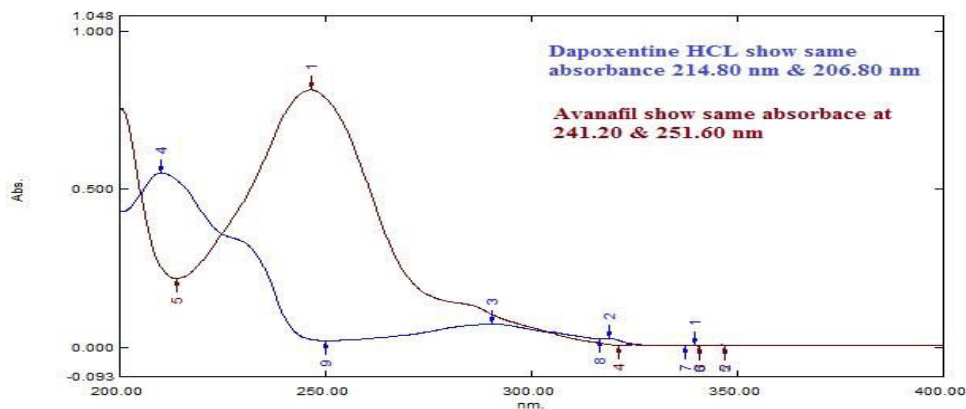


Figure no. 8: Zero order UV spectra of Avanafil and Dapoxetine HCL showing selection of wavelength for detection.

Standard curve

Avanafilat 214.80 nm and 206.80 nm		Dapoxetine HCL at 241.20 nm and 251.60 nm	
Concentration (µg/ml)	absorbance difference *	Concentration (µg/ml)	absorbance difference *
1	0.058	1	0.027
2	0.082	1.5	0.037
3	0.105	2	0.048
4	0.132	2.5	0.059
5	0.158	3	0.069
6	0.18	3.5	0.081

Table no. 11: STD curve for Avanafil and Dapoxetine HCL.

Conclusion:

The linearity range for Avanafil was found to be in the range of 1-6 µg/ml and for Dapoxetine HCL 1-3.5 µg/ml. Correlation co-efficient for calibration curve of Avanafil and Dapoxetine HCL was found to be 0.999 and 0.999 respectively.

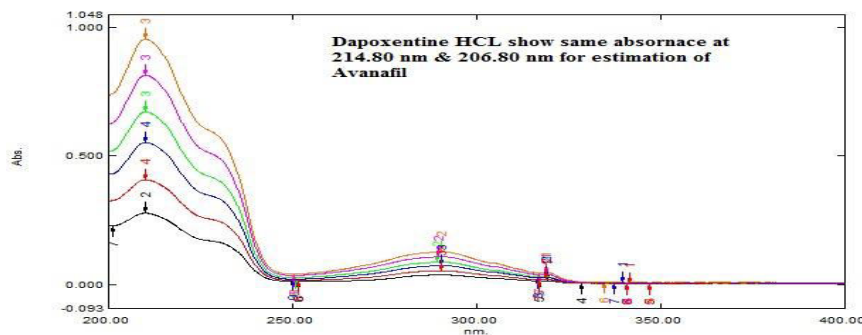


Figure no 9: Spectra for Avanafil and Dapoxetine HCL for different concentration at 214.80 nm and 206.80 nm where, Dapoxetine HCL has same absorbance and Avanafil has different absorbance.

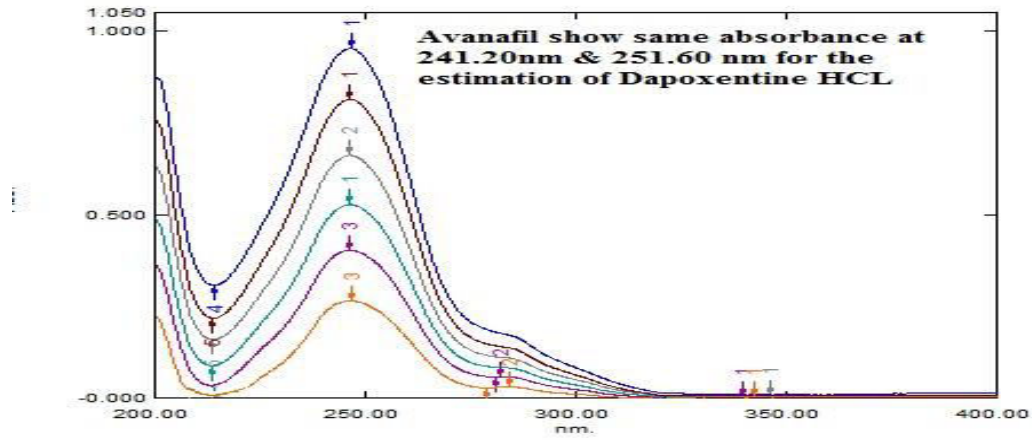


Figure no 10: Spectra for Avanafil and Dapoxetine HCL for different concentration at 241.20nm and 251.60 nm where, Avanafil has same absorbance and Dapoxetine HCL has different absorbance.

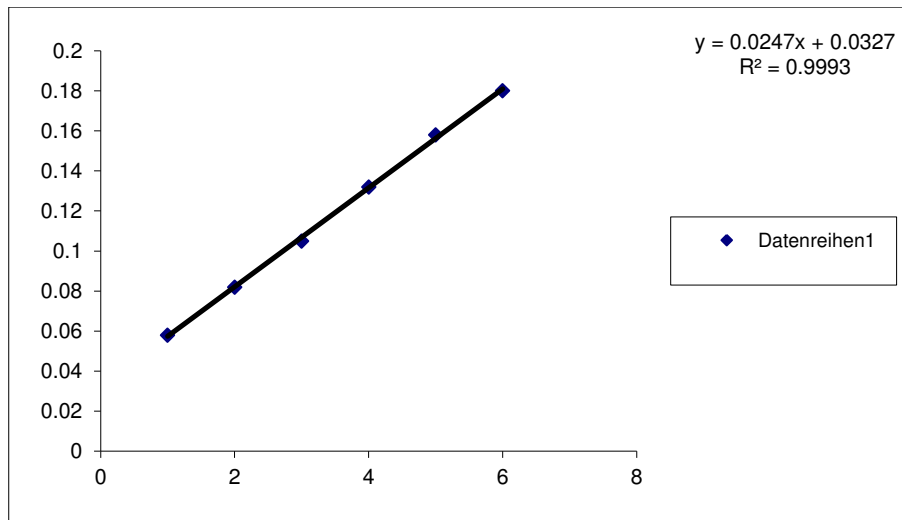


Figure 11: Calibration curve of Avanafil at 214.80-206.80 nm in 0.1N HCL.

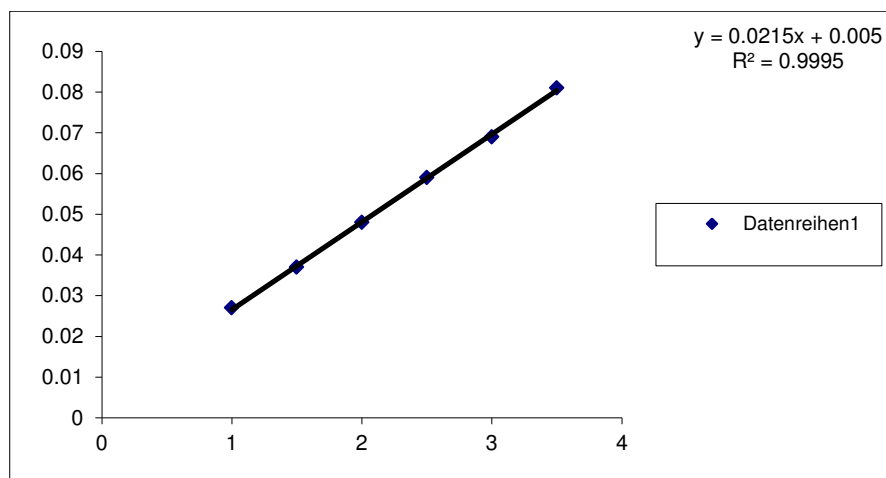


Figure 12: Calibration curve of Dapoxetine HCL at 241.20-251.60nm in 0.1N HCL.



Parameter	Avanafil at 214.80-206.80 nm	Dapoxetine HCL at 241.20-251.60nm
Regression lineEquation	$y = 0.0247x + 0.0327$	$y = 0.0215x + 0.005$
Correlation coefficient	0.9993	0.9995

Table 12: STD curve result.

**Validation**

**Linearity**

Avanafil at 214.80-206.80 nm		Dapoxetine HCL at 241.20-251.60nm	
Concentration (µg/ml)	Mean absorbance difference *±SD	Concentration (µg/ml)	Mean absorbance difference *±SD
1	0.058233 ± 0.000403	1	0.027517 ± 0.000376
2	0.082 ± 0.000443	1.5	0.037433 ± 0.000463
3	0.106333 ± 0.001211	2	0.048267 ± 0.000437
4	0.132833 ± 0.001722	2.5	0.059067 ± 0.000273
5	0.158 ± 0.00253	3	0.06895±0.000404
6	0.181 ± 0.002966	3.5	0.080583 ± 0.000631

Table no. 13: Linearity for Avanafil and Dapoxetine HCL.

**Precision**

**I. Repeatability**

Avanafil at 214.80-206.80 nm		Dapoxetine HCL at 241.20-251.60nm	
Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
5	0.158	3	0.0690
5	0.156	3	0.0687
5	0.155	3	0.0695
5	0.161	3	0.0693
5	0.157	3	0.0688
5	0.161	3	0.0684
<b>Mean</b>	0.158	<b>Mean</b>	0.0689
<b>SD</b>	0.0025	<b>SD</b>	0.0004
<b>% RSD</b>	1.6	<b>% RSD</b>	0.58

Table no. 14: Repeatability data for Avanafil at 214.80-206.80 nm and Dapoxetine HCL at 241.20-251.60nm.

**Discussion:** The % RSD for Repeatability of both the drugs was found to be less than 2. So, it was concluded that proposed method for estimation of Avanafil and Dapoxetine HCL is précised in nature.

**II. Intraday precision**

Avanafil Concentration (µg/ml)	Absorbance* ± S.D.	% RSD	Dapoxetine HCL Concentration (µg/ml)	Absorbance* ± S.D.	% RSD
3	0.128±0.0015	1.18	2.5	0.059±0.0006	1.14
4	0.157±0.0015	0.97	3	0.068±0.0003	0.44
5	0.184±0.0010	0.54	3.5	0.081±0.0002	0.30

\*n=3

**Table no.15: Intraday precision data for Avanafil at 214.80-206.80 nm and Dapoxetine HCL at 241.20-251.60nm**

**Discussion:** The % RSD for Repeatability of both the drugs was found to be less than 2 so, it was concluded that proposed method for estimation of Avanafil and Dapoxetine HCL is précised in nature

**III. Interday precision**

Avanafil Concentration (µg/ml)	Absorbance* ±S.D.	% RSD	Dapoxetine HCL Concentration (µg/ml)	Absorbance* ±S.D.	% RSD
3	0.131±0.0020	1.52	2.5	0.059±0.0002	1.75
4	0.156±0.0015	0.97	3	0.068±0.0004	0.66
5	0.185±0.0015	1.45	3.5	0.081±0.0004	0.49

\*n=3

**Table no 16: Interday precision data for Avanafil at 214.80-206.80 nm and Dapoxetine HCL at 241.20-251.60nm.**

**Discussion:** The % RSD for Repeatability of both the drugs was found to be less than 2 so, it was concluded that proposed method for estimation of Avanafil and is précised in nature

**Accuracy**

Level of recovery	Sample amount (µg/ml)	amount added (µg/ml)	amount recovered (µg/ml)	% recovery	AVG	SD	% RSD
80%	3	2.4	2.42	100.83	100.2767	0.479201	0.47
80%	3	2.4	2.4	100			
80%	3	2.4	2.4	100			
100%	3	3	3	100	99.88667	0.196299	0.19
100%	3	3	2.99	99.66			
100%	3	3	3	100			

120%	3	3.6	3.6	100	99.07333	1.605034	1.62
120%	3	3.6	3.6	100			
120%	3	3.6	3.5	97.22			

**Table no. 17: Accuracy (% Recovery) data for Avanafil.**

**Discussion:** Result obtained reveals that % recovery of Avanafil was within acceptance criteria given in ICH guideline.

Level of recovery	Sample amount (µg/ml)	amount added (µg/ml)	amount recovered (µg/ml)	% recovery	AVG	SD	%RSD
80%	3	2.4	2.4	100	99.86	0.242487	0.23
80%	3	2.4	2.4	100			
80%	3	2.4	2.39	99.58			
100%	3	3	3	100	102.2	3.810512	0.24
100%	3	3	3.2	106.6			
100%	3	3	3	100			
120%	3	3.6	3.6	100	99.07333	1.605034	1.62
120%	3	3.6	3.6	100			
120%	3	3.6	3.5	97.22			

**Table no. 18: Accuracy (% Recovery) data for Dapoxetine HCL.**

**Discussion:** Result obtained reveals that % recovery of Dapoxetine HCL was within acceptance criteria given in ICH guideline.

**Limit of Detection and Limit of Quantitation**

Parameters	Avanafil	Dapoxetine HCL
Mean Slope (n=6)	0.0329	0.0054
SD (n=6)	0.0006	0.0002
LOD (µg/ml)	0.069	0.13
LOQ (µg/ml)	0.21	0.42

**Table no. 19: LOD and LOQ data for Avanafil and Dapoxetine HCL.**

**Discussion:** The proposed method can detect and quantify small amount of drugs with precisely. So, it was concluded that the proposed method is very sensitive in nature.

BRAND NAME:	Drugs	Label Claim (mg)	Amount Found (mg)	% Label Claim*
OSENİ	Avanafil	100	101.82	101.82%
	Dapoxetine HCL	60	60.46	100.77%

**Table no. 20: Analysis of marketed formulation.**

**Discussion:** % Assay of Avanafil and Dapoxetine HCL was found in an acceptance limit so this method could be used for analysis of this combination.

**Conclusion:** Two simple, accurate, precise, reproducible and economical UV Spectrophotometric methods have been developed and validated for the estimation of Avanafil and Dapoxetine hydrochloride in pharmaceutical dosage form. All method validation parameters lie within its acceptance criteria as per ICH Q2 (R1) guideline so we can conclude that

methods are specific, linear, accurate and precise. Hence, it can be successfully used for the routine analysis of Avanafil and Dapoxetine hydrochloride in pharmaceutical dosage forms.

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