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# Simultaneous Estimation and Determination of Risperidone and Trihexyphenidyl by UV Spectrophotometric and RP-HPLC Method 

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#### Abstract

: A simple, rapid, economical, precise and accurate RP-HPLC method for simultaneous estimation of Cilostazole and Imipramine has been developed. A reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Cilostazole and Imipramine.. The separation was achieved by LC- 20 AT C18 ( $25 \mathrm{~cm} \times 0.46 \mathrm{~cm}$ ) Hypersil BDS column and Buffer ( pH 4.5 )-Methanol (20:80)as mobile phase, at a flow rate of $1 \mathrm{ml} / \mathrm{min}$. Detection was carried out at 222 nm . Retention time of Cilostazole and Imipramine were found to be 5.383 min and 3.153 min , respectively. The method has been validated for linearity, accuracy and precision. Linearity observed for Cilostazole $6-18 \mu \mathrm{~g} / \mathrm{ml}$ and for Imipramine $6-18 \mu \mathrm{~g} / \mathrm{ml}$. The percentage recoveries obtained for Cilostazole and Imipramine were found to be in range of $99.61 \pm 0.50$ and $99.78 \pm 0.65$ respectively. Developed method was found to be accurate, precise and rapid for simultaneous estimation of Cilostazole and Imipramine.. The proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial Combined dosage form.


Key words:Cilostazole, Imipramine, Simultaneous estimation, RP-HPLC Method, Validation.

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## 1.INTRODUCTION: ${ }^{[1-4]}$

Risperidone has exhibited good therapeutic efficacy against both positive and negative schizophrenic symptoms with low incidence of EPS.Risperidone (RIS) is a psychotropic agent belonging to the chemical class of benzisoxazole derivatives. Chemically it is 3-[2-[4-(6-fluoro-1, 2-benzisoxazol-3-yl)-1piperidinyl] ethyl]-6, 7, 8, 9-tetrahydro- 2-methyl-4H-pyrido [1, 2-a] pyrimidin-4-one.Trihexyphenidyl (THP) is an Antidyskinetics and antiparkinson drug whose IUPAC name is 1-cyclohexyl-1-phenyl-3-(1-piperidyl)-1-propanol1. THP is official in IP. IP suggest a titrimetric assay method for THP. The drug is used as an anticholinergic agent and anti Parkinson agent. Its mode of action is preventing the effects of Ach by blocking its binding to muscarinic cholinergic receptors at neuroeffector sites on smooth muscle, cardiac muscle, and gland cells; in peripheral ganglia; and in the central nervous system. Literature survey revealed that HPLC, UV and HPTLC methods5-28 have been reported for the estimation of RIS and THP individually and with other drugs in pharmaceutical dosage forms.RIS and THP are formulated together in the form of a tablet. Literature survey revealed no method reported for simultaneous determination of the two drugs. The present RP-HPLC method uses simple mobile phase ratio, higher
sensitivityand analysis will complete before 6 min. Therefore the present study was to determine both drugs concurrently by sensitive, accurate, rapid and precise in UV Spectrophotometric and RP-HPLC method.

## 2.MATERIALS AND METHODS

## Instruments and Reagents

A double beam UV-visible Spectrophotometer (Lab India, UV-3000+), attached to a computer software UV Win, with a spectral width of 2 nm , wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells. The chromatography was performed by a HPLC instrument (Shimadzu LC 2010 CHT) equipped with a UV-Visible detector, injector with Isocratic pump, SheisdoC $\mathrm{C}_{18}$ column (250* $4.6 \mathrm{~mm}, 5 \mu \mathrm{~m}$ ) and LC-solution software used. Analytical Balance and Ultra sonic cleaner were also used. RIS and TRI bulk powder was kindly gifted by Torrent Pharmaceuticals, Ahmedabadand Rhombus Pharmaceutical, Ahmedabad. HPLC grade methanol (Merck Ltd., Mumbai, India), HPLC grade Acetonitrile (Merck Ltd., Mumbai, India), Analytical grade Orthophosphoric acid and water were used.

### 2.1 RP-HPLC Method: ${ }^{[5-6]}$

## Chromatographic condition

The Chromatographic estimation was performed using following condition: Stationary phase: Sheisdo $\mathrm{C}_{18}$ column (250* $4.6 \mathrm{~mm}, 5 \mu \mathrm{~m}$ ) was used. Mobile Phase: Phosphate buffer (pH 3.5): Methanol (70:30 v/v) Flow rate: $1.0 \mathrm{ml} / \mathrm{min}$, Injection volume: $20 \mu \mathrm{~L}$, Detection: At 268nm with UV detector.Calibration curves for both RIS and TRI were plotted, and the corresponding regressions Equations were calculated.The chromatogram was stopped after separation achieved completely. Data related to peak like area, height, retention time, resolution etc.

### 2.2Preparation of Stock Solution

Accurately weighed RIS (20mg) and TRI (20mg) was transferred to a separate 100 ml volumetric flask and dissolved and diluted to the mark with methanol to obtained a standard stock solution having a concentration $\operatorname{RIS}(200 \mu \mathrm{~g} / \mathrm{ml})$ and TRI ( $200 \mu \mathrm{~g} / \mathrm{ml}$ ). From the above stock solution 0.25 ml was transferred to volumetric flask of 10 ml capacity. Volume was made up to the mark to give a solution containing $5 \mu \mathrm{~g} / \mathrm{ml}$ of RIS and TRI.

### 2.3 Preparation of Sample Solution

Powder from the mixed contents of 20 tablets, equivalent to 20 mg RIS and 20 mg TRI, was transferred accurately to a 100 ml volumetric flask and diluted to volume with methanol. The content was mixed with methanol ( 50 ml ), sonicated for 20 min to dissolve the drug ascompletely as possible. The volume was adjusted up to the mark with mobile phase 1.0 ml from this solution was taken in to a 10 ml volumetric flask and the volume was adjusted up to mark with mobile phase. The separation was done on a $\mathrm{C}_{18}$ column.

### 2.4 Calibration Curves

Calibration curves were constructed by plotting peak areas Vs concentrations of RIS and TRI and the regression equations were calculated. Calibration curves were plotted over a concentration range $5-25 \mu \mathrm{~g} / \mathrm{ml}$ for RIS and $5-25 \mu \mathrm{~g} / \mathrm{ml}$ TRI. Accurately measured standard working solutions $0.25,0.5,0.75,1.0, \& 1.25 \mathrm{ml}$ were transferred to a series of 10 ml of volumetric flasks and diluted to the mark with mobile phase. Aliquots ( $20 \mu \mathrm{~L}$ ) of each solution were injected under the operating chromatographic conditions.

## 3. UV Spectrophotometry: ${ }^{[7-11]}$

## i) Absorbance ratio method:

The absorbance of was measured at $268 \mathbf{n m}$ (Isoabsorptive point) and 258 nm ( $\lambda$ maxof TRI) for quantification of RIS and TRI. The amount of RIS and TRI present in the sample solution were determined by the substituted in the following equation to obtain the concentration,

## $C x=\{(Q M-Q y) /(Q x-Q y)\}^{*}(A 1 / a x 1)$

$C y=\{(Q x-Q M) /(Q y-Q x)\}^{*}(A 1 / a y 1)$

Where, CX and CY = concentration of Risperidone and Trihexyphenidyl respectively

A1 = Absorbance of mixture at Isoabsorptive point
ax1 = Absorptivity of Risperidone at Isoabsorptive point
ax2 $=$ Absorptivity of Risperidone at $\boldsymbol{\lambda}$ maxof Risperidone
ay1 = Absorptivity of Trihexyphenidyl at Isoabsorptive point
ay2 = Absorptivity of Trihexyphenidyl at $\lambda$ maxof Trihexyphenidyl

QX =(Absorptivity of Risperidone at 268 nm$)$ / (Absorptivity of Risperidone at 258 nm )

QY=(Absorptivity of Trihexyphenidyl at 258 nm ) / (Absorptivity of Trihexyphenidyl at 268 nm )

Qm= (Absorbance of the sample at 258 nm ) / (Absorbance of the sample at 268 nm )

## ii) Simultaneous equation method:

Weighed tablet powder equivalent to 20 mg of Risperidone and 20 mg of Trihexyphenidyl was transferred to 100 ml volumetric flask. Methanol was added, ultra-sonicated for 15 min . and volume made up to mark with methanol. The solution was filtered through Whattman filter paper No.41. The filtrate was further diluted with methanol to obtain concentration $5 \mu \mathrm{~g} / \mathrm{ml}$ of Risperidone and $5 \mu \mathrm{~g} / \mathrm{ml}$ Trihexyphenidyl. The concentration of both Risperidone and Trihexyphenidyl were determined by measuring the absorbances of sample at both wavelengths 280 nm and 258 nm . The concentrations of Risperidone and Trihexyphenidyl were calculated by solving these simultaneous equations.

$$
\begin{aligned}
& C x=\frac{A 2 \text { ay } 1-A 1 \text { ay } 2}{a x 2 \text { ay } 1-a x 1 \text { ay } 2}------(1) \\
& C y=\frac{A 1 \text { ax } 2-A 2 \text { ax } 1}{\text { ax2 ay } 1-\text { ax } 1 \text { ay } 2}------(2)
\end{aligned}
$$

Where, ax1 = Absorbance of Risperidone at 280 nm
ax2 = Absorbance of Risperidone at 258 nm
ay1 = Absorbance of Trihexyphenidyl at 258 nm
ay2 $=$ Absorbance of Trihexyphenidyl at 280 nm

## 3.RESULTS AND DISCUSSION

### 3.1 Validation of proposed method: ${ }^{[12-14]}$

Linearity and Range: The linearity is expressed in term of correlation co-efficient of linear regression analysis. The linearity of response for RIS and TRI was assessed by analysis of five independent levels of calibration curve in range of $\mathbf{5 - 2 5} \boldsymbol{\mu g} / \mathrm{ml}$ for RIS and $\mathbf{5 - 2 5} \boldsymbol{\mu \mathrm { g }} / \mathrm{ml}$ for TRI.

### 3.2 Precision

Result should be expressed as relative standard deviation (RSD) or co-efficient of variance.

## A.Repeatability

1 ml of combined working standard solutions ( $0.25 \mu \mathrm{~g} / \mathrm{ml}$ of RIS and $0.25 \mu \mathrm{~g} / \mathrm{ml}$ of TRI) were transferred into separate 10 ml volumetric flasks and diluted up to mark with mobile phase to get $5 \mu \mathrm{~g} / \mathrm{ml}$ of RIS and TRI. Each concentration was prepared and analyzed.

The peak area obtained with each solution was measured and \% R.S.D was calculated.

## B. Intra-day precision

Mixed solutions containing $5,15,25 \mu \mathrm{~g} / \mathrm{ml}$ RIS and 5,15 , $25 \mu \mathrm{~g} / \mathrm{ml}$ TRI were analyzed on the same day and \% R.S.D was calculated.

## C. Inter-day precision

Mixed solutions containing $5,15,25 \mu \mathrm{~g} / \mathrm{ml}$ RIS and 5,15 , $25 \mu \mathrm{~g} / \mathrm{ml}$ TRI were analyzed on different days and \% R.S.D was calculated.

### 3.3 Accuracy

The \% recovery experiment was performed by the Standard Addition Method. Known amounts of standard solutions of RIS (4, 5, $6 \mu \mathrm{~g} / \mathrm{ml}$ ) and TRI (4, 5, $6 \mu \mathrm{~g} / \mathrm{ml}$ ) were added at 80,100 and $120 \%$ level to prequantified sample solutions of RIS ( $5 \mu \mathrm{~g} / \mathrm{ml}$ ) and TRI ( $5 \mu \mathrm{~g} / \mathrm{ml}$ ).Area of peak obtained with each solution was measured for RIS and TRI.

The amount of RIS and TRI was calculated at each level and \% Recoveries were computed.

### 3.4 LOD and LOQ

The LOD was estimated from the 5 calibration curves. The LOD may be calculated as

LOD $=3.3 \times(S . D /$ Slope $)$
Where, S.D = Standard deviation of the Y - intercepts of the 5 calibration curves.

Slope $=$ Mean slope of the 5 calibration curves.
The LOQ was estimated from the calibration curves used to determine method linearity. The LOQ may be calculated as

LOQ $=10 \times(S . D / S l o p e)$

Where, SD = Standard deviation of the Y - intercepts of the 5 calibration curves.

Slope $=$ Mean slope of the 5 calibration curves.

### 3.5 Robustness:

The solution containing concentration ( $20 \mu \mathrm{~g} / \mathrm{ml}$ ) of RIS and ( $20 \mu \mathrm{~g} / \mathrm{ml}$ ) of TRI was analyzed in different pH , Mobile phase and Flow rate and the peak area obtained with each solution was measured and \% R.S.D was calculated.

Acceptance criteria: \% R.S.D should be less than 2.

## CONCLUSION:

RP-HPLC, Simultaneous Equation and Absorbance Ratio method has been developed and validated for the determination of Risperidone and Trihexyphenidyl in pharmaceutical dosage form. The method was found to be specific as there was no interference of excipients and impurities. The proposed method was found to be simple, linear, accurate, precise. Hence, it can be successfully used for the routine analysis of Risperidone and Trihexyphenidyl from pharmaceutical dosage form.

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TABLES AND FIGURES:
FOR RP-HPLC:


Fig. 1 RP-HPLC Chromatogram of RIS and THP


Fig. 2 Linearity chart of Risperidone


Fig. 3 Linearity chart of Trihexyphenidyl

## FOR UV SPECTROPHOTOMETRIC:

Q - Absorbance ratio method :


Fig 7. Calibration curve of Risperidone


Fig 8. Calibration curve of Trihexyphenidyl



Fig 5.Calibration Curve of Isoabsorptive Point at 268 nm
> Simultaneous equation method:


Fig 6. Overlain spectra of Risperidone and
Trihexyphenidyl

TABLE 2 : Recovery data for RIS
\(\left.$$
\begin{array}{lllll}\hline & & \begin{array}{l}\text { Amount } \\
\text { Standard } \\
\text { added (\%) }\end{array} & \begin{array}{l}\text { of }\end{array} & \begin{array}{l}\text { Amount } \\
\text { Standard } \\
\text { Recovered } \\
\text { (\%) }\end{array}\end{array}
$$ \begin{array}{l}Recovery of <br>

RIS (\%)\end{array}\right]\)| Spike <br> (n=3) | I | 4 | 3.981 |
| :--- | :--- | :--- | :--- |

TABLE 3 : Recovery data for TRI


Table 5: Regression Analysis Data and Summary of Validation Parameter for the Proposed Method

| Parameters | Q-Absorbance RatioMethod |  | Simultaneous equation method RIS at 280 nm |
| :---: | :---: | :---: | :---: |
|  | TRI at 258 nm | RIS at 268 nm |  |
| Concentration range ( $\mu \mathrm{g} / \mathrm{ml}$ ) | 5-25 | 5-25 | 5-25 |
| Regression | $Y=0.034 x+$ | $Y=0.019 x+$ | $Y=0.026 \mathrm{x}+$ |
| Equation | 0.013 | 0.009 | 0.009 |
| Slope (m) | 0.034 | 0.019 | 0.026 |
| Intercept (C) | 0.013 | 0.006 | 0.009 |
| Regression coefficient ( $\mathbf{r}^{2}$ ) | 0.999 | 0.998 | 0.999 |
| InterdayPrecision | 0.344 | 0.666 | 1.659-1.023 |
| ( $\mathrm{n}=3$ ) $\pm$ \% R.S.D. | 0.990 | 1.408 |  |
| IntradayPrecision | 0.226 | 0.849 | 1.492-0.757 |
| $(\mathrm{n}=3) \pm$ \% R.S.D. | 1.735 | 1.385 |  |
| LOD ( $\mu \mathrm{g} / \mathrm{ml}$ ) | 0.074 | 0.276 | 0.105 |
| LOQ ( $\mu \mathrm{g} / \mathrm{ml}$ ) | 0.224 | 0.837 | 0.320 |
| Mean \%Recovery | 99.53 | 99.39 | 98.80-101.46 |
|  | 100.24 | 101.91 |  |
| \% Assay | 97.00 | 96.08 | 98.05 |



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