NOVEL METHOD FOR CHROMATOGRAPHIC DETERMINATION OF SETRALINE AND DOXOFYLLINE IN FORMULATION BY USING RP-HPLC

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ABSTRACT
A simple, rapid, selective, sensitive, linear, precise and accurate NOVAL METHOD FOR CHROMATOGRAPHIC DETERMINATION OF SETRALLINE AND DOXOFYLLINE. Tablet dosage form separation of drug was achieved on a reversed phase symmetry C18 column (150mm x 4.6mm) phosphate buffer was pH 3.5 & the mobile phase was optimized with consist of Acetonitrile, phosphate buffer mixed in the ratio of 30:70% v/v. The solution was chromatographed at constant flow rate of 1.0 ml / min. The UV detection wavelength was 234nm & 10 µm of sample was injected. The linearity ranges of SERTRALINE & DOXOFYLLINE were found to be 0.58µm from 1.77µg/ml of SERTRALINE & DOXOFYLLINE 0.27 & 0.82µg/ml. Linear regression coefficient was more than 99-100% the value RSD is ranged from 0.37-0.53% indicating accuracy & precision of the method. LOD & LOQ were found to be within limit. The method was validated as per ICH guidelines for its sensitivity, linearity, accuracy & precision. The method was successfully employed for routine quality control analysis SERTRALINE & DOXOFYLLINE in its pharmaceutical formulations.

Keywords: Novalmethod for Chomatographic Determination of Setralline and Doxofylline.

INTRODUCTION
Pharmaceutical analysis plays a vital role in the Quality Assurance and Quality control of bulk drugs.\textsuperscript{[1]} Analytical chemistry involves separating, identifying, and determining the relative amounts of components in a sample matrix. Pharmaceutical analysis is a specialized branch of analytical chemistry. Pharmaceutical analysis derives its principles from various branches of sciences like physics, microbiology, nuclear science and electronics etc.
Qualitative analysis reveals the chemical identity of the sample. Quantitative analysis establishes the relative amount of one or more of these species or analytes in numerical terms. Qualitative analysis is required before a quantitative analysis can be undertaken. A separation step is usually a necessary part of both qualitative and quantitative analysis. The results of typical quantitative analysis can be computed from two measurements. One is the mass or volume of sample to be analyzed and second is the measurement of some quantity that is proportional to the amount of analyte in that sample and normally completes the analysis.

CHROMATOGRAPHY

Chromatography\(^3\) is a separation of mixture into individual components using a stationary phase and a mobile phase. This may be regarded as an analytical technique employed for the purification and separation of organic and inorganic substances. There are various advanced chromatographic techniques, widely used for the estimation of multicomponent drugs in their formulations. The various chromatographic techniques are

- High Performance Liquid Chromatography.
- High Performance Thin Layer Chromatography.
- Gas Chromatography.

1.1.1. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

HPLC is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase. The technique of high performance liquid chromatography is so called because of its improved performance when compared to column chromatography. Advances in column technology, high-pressure pumping system and sensitive detectors have transformed liquid column chromatography into high speed, efficient, accurate and highly resolved method of separation.

The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

- Greater sensitivity (various detectors can be employed).
- Improved resolution (wide variety of stationary phases).
- Reusable columns (expensive columns but can be used for many analysis).
- Ideal for the substances of low viscosity.
- Easy sample recovery, handling and maintenance.
Instrumentation leads itself to automation and quantification (less time and less labour).
Precise and reproducible.
Integrator itself does calculations.

Based on Modes of Chromatography
- Normal phase chromatography.
- Reverse phase chromatography.

EXPERIMENTAL AND RESULTS AND DISCUSSION

INSTRUMENTS USED

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Name of Instrument</th>
<th>Instrument Model</th>
<th>Name of manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>UV-Visible double beam spectrophotometer</td>
<td>UV 1800</td>
<td>Elico India</td>
</tr>
<tr>
<td>2</td>
<td>HPLC</td>
<td>1575</td>
<td>Hitachi LaChrome</td>
</tr>
<tr>
<td>3</td>
<td>Ultra sonicator</td>
<td>-------</td>
<td>Entech electronics limited</td>
</tr>
<tr>
<td>4</td>
<td>Melting point apparatus</td>
<td>-------</td>
<td></td>
</tr>
</tbody>
</table>

CHEMICALS / REAGENTS USED

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Name</th>
<th>Specifications</th>
<th>Manufacturer/Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Doubled distilled water</td>
<td>----</td>
<td>Sd fine-Chem ltd; Mumbai</td>
</tr>
<tr>
<td>2.</td>
<td>Methanol</td>
<td>99.9% A.R.</td>
<td>Loba Chem; Mumbai</td>
</tr>
<tr>
<td>3.</td>
<td>Dipotassium hydrogen phosphate</td>
<td>96% L.R.</td>
<td>Sd fine-Chem ltd; Mumbai</td>
</tr>
<tr>
<td>4.</td>
<td>Acetonitrile</td>
<td>99.9% HPLC</td>
<td>Loba Chem; Mumbai</td>
</tr>
<tr>
<td>5.</td>
<td>Potassium dihydrogen orthophosphate</td>
<td>99.9% L.R.</td>
<td>Sd fine-Chem ltd; Mumbai</td>
</tr>
<tr>
<td>6.</td>
<td>orthophosphoric acid</td>
<td>99.9% L.R.</td>
<td>Sd fine-Chem ltd; Mumbai</td>
</tr>
</tbody>
</table>

CHARACTERIZATION OF SERTRALINE & DOXOFYLLINE

Solubility of Sertraline
The solubility of drug sample was determined according to I.P. 1996. [38]

Two 10 ml and one 250 ml volumetric flasks were taken.

Flask 1: 10 mg of Sertraline was accurately weighed and transferred to 10 ml volumetric flask. 0.1 ml of water was added into it. The contents were mixed for one minute. The drug was slightly dissolved. Again 0.1 ml of water was added into the volumetric flask. The contents were mixed for one minute. The solubility state was noted.
Flask 2: 10mg of Sertraline was accurately weighed and transferred to 10 ml volumetric flask. 0.1 ml of methanol was added into it. The contents were mixed for one minute. The drug was slightly dissolved. Again 0.1 ml of methanol was added into the volumetric flask. The contents were mixed for one minute. The solubility state was noted.

Flask 3: Accurately weighed Sertraline (10 mg) was transferred to 250 ml volumetric flask. Added 10 ml of acetonitrile to it. Mixed the solution for one minute. The drug could not dissolve. Added more 90 ml of acetonitrile to the volumetric flask. Mixed the solution for two minutes. The drug could not dissolve. Added more 100 ml of water to the volumetric flask. Mixed the solution for two minutes. The solubility state was noted.

Solubility of Doxofylline
The solubility of drug sample was determined according to I.P. 1996[38].
Two 10 ml volumetric flasks were taken.

Flask 1: Accurately weighed Doxofylline (10 mg) was transferred to 10 ml volumetric flask. Added 0.1 ml of water to it. Mixed the solution for one minute. The drug could not dissolve. Added more 0.4 ml of water to the volumetric flask. Mixed the solution for two minutes. The solubility state was noted.

Flask 2: Accurately weighed Doxofylline (10 mg) was transferred to 10 ml volumetric flask. 0.1 ml of methanol was added into it. The contents were mixed for one minute. The drug was slightly dissolved. Again 0.1 ml of methanol was added into the volumetric flask. The contents were mixed for one minute. The solubility state was noted.

RESULT AND DISCUSSION
Sertraline was found to be freely soluble in water and soluble in acetonitrile & methanol. Doxofylline was found to be soluble in water and soluble in methanol & acetonitrile.

METHOD DEVELOPMENT AND ITS VALIDATION FOR SIMULTANEOUS ESTIMATION OF SERTRALINE & DOXOFYLLINE BY RP-HPLC IN COMBINATION TABLET DOSAGE FORM
Selection of wavelength
The $\lambda_{\text{max}}$ of the two ingredients i.e. Sertraline & Doxofylline, were found to be 222 nm and 273 nm respectively in methanol as solvent system. the isobestic point for the drugs were found at 273 nm.
4.6.2 Preparation of standard solution of Sertraline

10 mg of Sertraline was weighed accurately and transferred into 100 ml volumetric flask. About 10 ml of HPLC grade methanol was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 100 μg/ml of Sertraline.

4.6.3 Preparation of standard solution of Doxofylline

10 mg of Doxofylline was weighed accurately and transferred into 100 ml volumetric flask. About 10 ml of HPLC grade methanol was added and sonicated to dissolve. The volume was made up to the mark with same solvent. The final solution contained about 100 μg/ml of Doxofylline.

Preparation of mix. Standard solution of Sertraline & Doxofylline

Accurately weighed 100 mg of Sertraline and 100 mg of Doxofylline were transferred to two different 100 ml volumetric flask. About 40 ml of mobile phase was added and sonicated to
dissolve. The volume was made up to mark with same solvent. Then 0.5, the detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

\[
L.O.D. = 3.3(\text{SD}/S).
\]

\[
L.O.Q. = 10(\text{SD}/S)
\]

Where, SD = Standard deviation of the response  
S = Slope of the calibration curve

**RESULT AND DISCUSSION**

The LOD was found to be 0.32 μg/ml and 1.44 μg/ml and LOQ was found to be 0.96 μg/ml and 4.32 μg/ml for Sertraline & Doxofylline respectively which represents that sensitivity of the method is high.

![Chromatograms for LOD](image1)

![Chromatograms for LOQ](image2)
6 System Suitability Parameter

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters were established. The data are shown in Table 39.

Table 39: Data of System Suitability Parameter.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parameter</th>
<th>Limit</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Resolution</td>
<td>Rs &gt; 2</td>
<td>3.15</td>
</tr>
<tr>
<td>2</td>
<td>Asymmetry</td>
<td>T ≤ 2</td>
<td>Sertraline =0.14 Doxofylline =0.19</td>
</tr>
<tr>
<td>3</td>
<td>Theoretical plate</td>
<td>N &gt; 2000</td>
<td>Sertraline =3971 Doxofylline= 4861</td>
</tr>
</tbody>
</table>

ASSAY OF SERTRALINE & DOXOFYLLINE IN DOSAGE FORM

Assay was performed as described in previous chapter. Results obtained are tabulated below:

Assay of SERTRALINE & DOXOFYLLINE tablets.

<table>
<thead>
<tr>
<th>Brand name of tablets</th>
<th>Labeled amount of Drug (mg) Sertraline &amp; Doxofylline</th>
<th>Mean (±SD) amount (mg) found by the proposed method (n=6)</th>
<th>Mean (± SD) Assay (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxoder (Wonder Healthcare)</td>
<td>50, 400</td>
<td>50.04 (±0.13) 400.53 (±0.09)</td>
<td>100.08 (±0.39) 100.1325 (±0.42)</td>
</tr>
</tbody>
</table>

The assay of Doxoder tablets containing Sertraline was found to be 100.08% Doxofylline was found to be 100.1325%.

RESULT AND DISCUSSION

To develop a precise, linear, specific RP-HPLC method for analysis of Sertraline & Doxofyllinedifferent chromatographic conditions were applied & the results observed are presented in the thesis.

Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution.

In case of RP-HPLC various columns are available, but here develop Sil, C-18, V size (150mm*4.6mmØ) column was preferred because using this column peak shape, resolution and absorbance were good.
Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl). Sertraline was found to be soluble in water and soluble in acetonitrile & methanol. Doxofyllinewas found to be insoluble in water and soluble in methanol & acetonitrile.

Detection wavelength was selected after scanning the standard solution of drug over 200 to 800nm. From the U.V spectrum of Doxofylline & Sertralineit is evident that most of the HPLC work can be accomplished in the wavelength range of 215-290 nm conveniently. Further, a flow rate of 1.0 ml/min & an injection volume of 20 µl were found to be the best analysis.

The result shows the developed method is yet another suitable method for assay which can help in the analysis of Doxofylline & Sertralinein different formulations.

CONCLUSION

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Sertraline & Doxofylline API.

Based on peak purity results, obtained from the analysis of samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Sertraline & Doxofyllineindicated that the developed method is specific for the estimation of Sertraline & Doxofylline.

Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

REFERENCE

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