ANALYTICAL METHOD VALIDATION OF TABLET DOSAGE FORM OF OPIPRAMOL HCL

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Abstract:
The objective of the work is to validate the technique as an internal method is the primary goal of the method validation of opipramol HCl as a tablet dosage form. Due to the fact that the tablet dosage form is not listed in any pharmacopeia, a proper technique has been developed for future research, and a sample has been examined octadecylsilane (C18) column shim pack shimadzu stationary Phase. Phosphate buffer, acetonitrile, and methanol in a 40:40:20 ratio with orthophosphoric acid to set the pH to 4. Run at a 1 ml/min flow rate. With a UV-Visible Detector at 210 nm wavelength and room temperature, quantification is accomplished. Over a sample concentration range of 80 ppm to 120 ppm, the calibration curve was linear. The limits of detection and quantification were determined to be 11.26 µg/ml and 34.12 µg/ml respectively. There was no evidence of chromatographic interference from excipients in the chromatogram. In order to determine the amount of opipramol in a formulation, the technique is used. The procedure is therefore verified.

Keywords: Opipramol, Analytical method validation, reversed-phase HPLC, Assay and Area vs concentration.

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Introduction:
According to previous studies, opipramol Dihydrochloride is a relatively powerful sigma ligand with prominent D2-,5-HT2-, and H1-blocking potential and high affinity to sigma1 and reduced affinity to sigma2 sites. Opipramol is a novel anxiolytic and antidepressant medication. It is a psychoactive medication frequently used to treat somatoform, generalized anxiety disorder and anxious depressive disorders. The biphasic activity at first causes rapid relief of stress, anxiety, and insomnia [1][2].

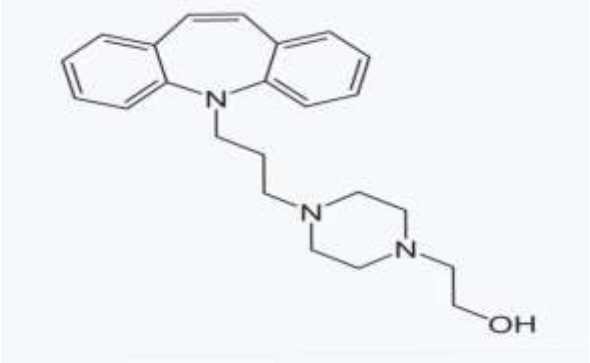

Fig. 1: Structure of Opipramol Dihydrochloride

Validation: The written proof from the validation process offers a high level of assurance for the desired outcome with guaranteed conformity. In the pharmaceutical industry, the phrase "validation" is frequently employed. The term "legally defined" is derived from the word "valid or validity." The Food and Drug Administration (FAD) first suggested the validation concept to raise the caliber of pharmaceutical items in the middle of the 1970s. Since many different processes, techniques, or activities are validated to examine and enhance their quality[3].

Types of validation[4][5][6][7]:
Validation is divided into following subsections which include:
1. Analytical method validation
2. Process validation
3. Cleaning validation
4. Equipment validation

1. Analytical method validation: The purpose of analytical validation is to verify that the selected analytical procedure will give reliable results that are adequate for the intended purpose. There are different parameters which come under analytical method validation. These are as follows:

- Accuracy
- Precision
- Repeatability
- Reproducibility
- Specification
- Linearity
- Range
- Detection limit

2. Process validation: This type of validation demonstrates documented proves, which carries a higher degree of surety that the process will consistently produce a product which meets all the predetermined quality characteristics and specifications. The process validation also assures the repeatability of the process and decreases the risk of manufacturing problems which lead to an increase in output of predetermined quality.
On the bases of the stage of production under process validation, it can be of four types which are as follow:

- Prospective validation
- Concurrent validation
- Retro specific validation
- revalidation

3. Cleaning validation: Cleaning validation provides documented set up with a high degree of surety that particular system/equipment or part of equipment is consistently clean-up to predetermined quality and acceptable limits. Pharmaceutical products are contaminated by variety of substances such as lubricants, airborne materials, prepared product residues, and microbes. Hence, an adequate cleaning procedure plays an important role to prevent contamination and cross contamination.

4. Equipment validation: Equipment validation is established documented set up that proves any equipment works correctly and leads to accepted and accurate results (predetermined result). The process of equipment validation is based on the principle that equipment must be designed, constructed, maintained, and adapted to perform the operations which are to be carried out.

Equipment's are the basic component of pharma industries; therefore, before performing a process in pharma industries, it becomes primary important to issue equipment validation (documented evidences of equipment),

Experimental section

Materials and methods:

Apparatus:

Shimadzu Liquid chromatography (LC 2010CHT HPLC System) having UV- Visible detector and Lab Solution Software , octadecylsilane (C18) 250 x 4.0 mm 5 micron shim pack shimadzu stainless steel Column As stationary Phase , Shimadzu Analytical Balance AP Series Sonicator (Precioustech) , Digital Ph Meter (Hanna instruments) , Glassware's like Pipette , Beaker , Volumetric Flask , etc. From Supertek. Syringe , nylon filter 0.45 micron size , Milli Q Water System (HPLC Grade Water System) .

Chemicals:

Opipramol Working reference Standard is used From Concern Pharma Ltd . Potassium di hydrogen orthophosphate AR , Acetonitrile HPLC grade , Methanol HPLC grade , Milli Q Water , Orthophosphoric Acid , 100 mg tablet (jasdep-100 Tablet From Concern Pharma Ltd).

Chromatographic conditions:

Detection : λ max: 210 nm
Flow rate: 1 ml / min
Column: C18 – 250 x 4.6, 5µ
Injection volume - 20 µl
Temperature: 35.00C

Preparation of mobile phase [8]:

Phosphate buffer is the mobile phase: Methanol: Acetonitrile Ph 4 in the ratio of 40:40:20. 2.72 gram of potassium di hydrogen orthophosphate is dissolved in 400 ml of Milli Q water (HPLC Grade Water) in a 1 litre beaker to create phosphate buffer. Following the mixing of the solute particles in the beaker using an ultra sonicator, 400 ml of acetonitrile and 200 ml of methanol are added. Then the mixture is completely degassed by placing the beaker in an ultra sonicator for up to 15 to 20 minutes. After degassing, the mixture is placed on a pH meter and continuously stirred with a magnetic stirrer. Next, the mixture is dipped into the glass probe of the PH meter, and the pH is then maintained at 4. Adding orthophosphoric acid to the mixture In dropsThe mixture is filtered using a vacuum filtering assembly with a filter of 0.4 mm size once the Ph is maintained. Following filtering, the mobile phase is prepared.

Preparation of sample solution:

Accurately measure 20 tablets and grind them into a fine powder. Transfer the measured amount, which is approximately 100 mg of opipramol 2HCl, to a volumetric flask with a capacity of 100 ml. Make up the volume with Mobile phase and additional material after adding 30 ml of it and sonicating for 5 minutes. In a 10 ml volumetric flask, dilute 1 ml of the first dilution with the mobile phase to the desired volume.

Preparation of standard solution:

Transfer 50 mg of Opipramol 2HCl WRS to a 50 ml volumetric flask after precisely weighing the dosage. Shake the mobile phase to dissolve after adding about 30 milli litres to make up the volume. Put 1 ml of the first dilution into a volumetric flask and add 10 ml of the mobile phase to make the volume.

Analytical method validation:

The parameter for performing method validation are linearity, Accuracy , recovery , specificity , limit of detection , limit of detection , limit of quantification and robustness are studied .

Result and discussion:

Linearity :

Different sample concentrations, such as 32ppm, 36ppm, 40ppm, 44ppm, and 48ppm, which are 80%, 90%, 100%, 110%, and 120% of the main sample concentration, are prepared. The concentration vs. area plot was found to be linear, and the correlation coefficient r2 was found to be 0.9992. To estimate the average area of different triplicate injections, triplicate injections of various concentrations are done and mentioned in Table no.1

Concentration	1st injection area	2nd injection area	3rd injection area	Average area	Std deviation	%RSD
32ppm (80%)	1634092	1633083	1633523	1633566	505.8725136	0.030967375
36ppm (90%)	1839533	1829178	1839978	1836236.333	6116.388504	0.333093752
40ppm (100%)	2036881	2039622	2047354	2041286	5431.093107	0.266062374
44ppm (110%)	2246439	2245184	2242677	2244766.667	1915.407615	0.085327693
48ppm (120%)	2454791	2454551	2462975	2457439	4795.818178	0.195155126
						0.182121264

Retention time of different concentration injections are mentioned below in Table no.2

Concentration	Injections	Area	Retention Time	%RSD	Avg retention time
32ppm	1st	1634092	9.839	0.018	9.840
	2nd	1633083	9.840		
	3rd	1633523	9.842		
36ppm	1st	1839533	9.836	0.009	9.836
	2nd	1829178	9.835		
	3rd	1839978	9.836		
40ppm	1st	2036881	9.832	0.032	9.836
	2nd	2039622	9.838		
	3rd	2041286	9.837		
44ppm	1st	2246439	9.833	0.031	9.835
	2nd	2245184	9.834		
	3rd	2242677	9.838		
48ppm	1st	2454791	9.832	0.016	9.834
	2nd	2454551	9.833		
	3rd	2462975	9.835		

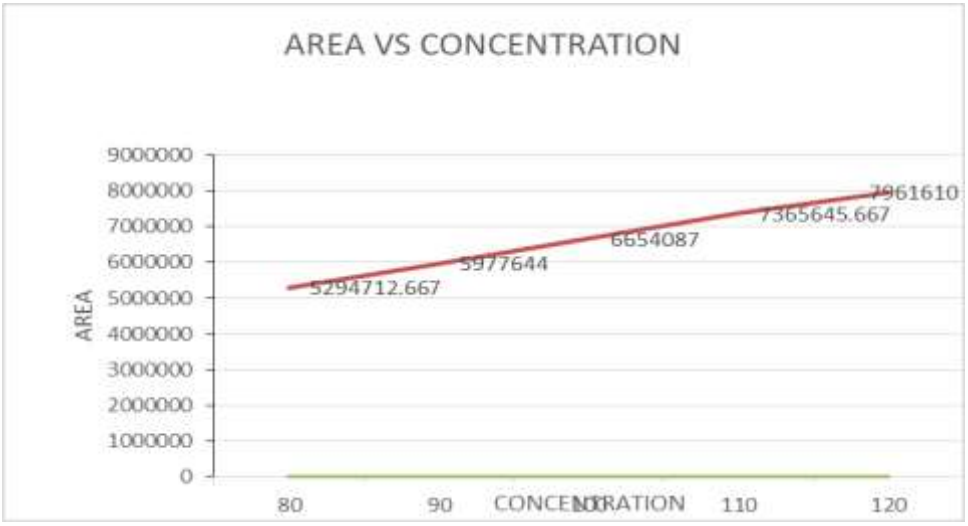

Figure no.2

Accuracy:

Average recoveries of opipramol triplicates 80% , 90% , 100%, 110%, 120% is in acceptable criteria (as mentioned in Table 2). Excellent recovery with low relative standard deviation is observed .which means that the method can be succesfully applied for the assay determination in potent opipramol Tablet .

Specificity :

Specificity is deifned as a specific precision for a well developed method that can be analysed again at same conditions and parameters . The other comonents are used to check the interference of those and the main API used . So, the placebo used in formation of tablet as per average weight of tablet is used as a refernce to check whether its presence can effect the formulation or not .I it effects than one can check the area difference , amplitude of peaks obtained , might be slight change in retention time etc.

Method Precision[9] :

Six replicate injections of sample solution are injectedto check whether the applied method is suitable or not. The % RSD was found below 2%. And results obtained can be seen in table 1 & 2.The indication of correctively of the method is precised by repeatedly applying multipe injections.

Stability of sample and standard solution [10][11]:

After succesfull running of batch on day 1 . the sample and standard are preserved at room temperature to get reinjected on day 2 , and after 24 hour same sample set is runned after completion of batch we observed that RSD is below 2 % , this indicated that sample and standard is stable on day .

Limit of Detection and limit of Quantification

LOD and LOQ were calculated by using equations designated by ICH. LOD and LOQ values were

calculated as signal-to noise ratio of 3:1 and 10:1 respectively.

$LOD = 3.3 \times \sigma/S$, $LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Value of slope in Plot 1 is 67218 and standard deviation is 102585.4999

So , after applying values in formula , we can able to LOD and LOQ.

The LOD is found to be 11.26 µg/ml and LOQ is found to be 34.12 µg/ml.

Robustness:

Robustness of the analytical method is checked by performing minor variation in method parameters and mobile phase preparation . in method parameters the column oven temperature is varied $\pm 2^{\circ}c$ and in mobile phase preparation the Ph is varied ±0.5 .

Hence , No major difference in the results is observed with minor changes in Retention time of sample and standard . So the method is validated for robustness.

Conclusion :

A simple, sensitive isocratic method is developed on RP-HPLC with UV Visible Detector . The method is validated for Linearity, Accuracy, Recovery, Specificity, precision, stability, LOD, LOQ and Robustness.

Opipramol Showing excellent detection at 210 nm in C18 – 250 x 4.6, 5µ Column. Sample is runned at flow rate of 1ml/min. with injection volume of 20µl. Uv- visible Detector's detection found efficient for opipramol sample at 210nm. The calibration curves found to be linear up to 32 ppm to 48 ppm concentration with r^2 value of 0.9992 . Method is found to be specific without interference of excipients with peak of opipramol. Method gets précised by injecting multiple replicate injections with RSD below 2%. Sample is also tested for stability up to 24 hours . LOD and LOQ values were calculated as signal-to noise ratio of 3:1 and 10:1 respectively. LOD and LOQ values are 11.26 µg/ml and 34.12 µg/ml. The result of analysis states that the method is highly Reproducible and cab be employed for routine testing of opipramol in dosage forms

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