Development and Validation of a Simple and Rapid Reversed Phase Liquid Chromatography Method for Estimation of Pregabalin from Equipment Surfaces Used for Pharmaceutical Manufacturing

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Abstract

A simple and rapid reversed phase HPLC method was developed and validated for estimation of pregabalin from the surfaces of equipment used for pharmaceutical manufacturing. The chromatographic separation was achieved on Waters Symmetry Shield RP18 column (5.0 µm, RP18, 250 mm x 4.6 mm) at 40°C by using isocratic elution using methanol and sodium di hydrogen phosphate monohydrate buffer (pH adjusted to 6.30 with NaOH solution; 0.01M) at flow rate of 0.8 ml/min. UV detection was performed at 200 nm. Water was used as swabbing solvent to extract the drug residues from the stainless steel surface. Texwipe swabs (polyester swab) were used to remove the drug from the stainless steel surface. The method was validated for system suitability, specificity recovery, limit of detection, limit of quantification, linearity, ruggedness and robustness. The recovery values from stainless steel surface were found more than 93.5%. The limit of detection and limit of quantification were 0.20 µg/ml and 0.39 µg/ml respectively. Method was found precise at concentration level 0.3917 µg/ml. Method was found linear from 0.39 µg/ml to 2.7979 µg/ml. The coefficient of correlation was observed 0.9999.

Introduction

The one of the objective of good manufacturing practices include the prevention of possible contamination and cross contamination of pharmaceutical products. Contamination may happen due to a variety of things but one of the major reasons is contamination due to previous product manufactured in the same equipment. Such kind of contaminations may impact the safety of the product due to which a lot of recalls happened. This kind of contamination can be prevented by adopting an adequate cleaning procedure that needs validation to ensure decontamination, particularly in case of multi product facilities. Cleaning validation is not necessary for cleaning between batches of same product. For cleaning validation, a cleaning validation protocol is required which describes mainly equipment cleaning methodology, sampling and analytical method [1-3].

Pregabalin is an anticonvulsant drug used for neuropathic pain and as an adjunct therapy for partial seizures with or without secondary generalization in adults. It has also been found effective for generalized anxiety disorder and is (as of 2007) approved for this use in the European Union and Russia [4,5]. It is effective at treating some causes of chronic pain such as fibromyalgia but not others. It is considered to have a low potential for abuse, and a limited dependence liability if misused, but is classified as a Schedule V drug in the U.S. Schedule V drugs are the drug or other substances which has a low potential for abuse relative to the drugs or other substances in schedule IV.

Pregabalin is available in capsule and oral solution dosage forms, marketed by Pfizer under the trade name Lyrica. Its capsule dosage form is available in multiple strengths as 25, 50, 75, 100, 150, 200, 225, and 300 mg/capsule. Its oral solution dosage form is available in 20 mg/ml strength. Pfizer described in an Securities and Exchange Commission Filing (SEC) that the drug could be used to treat epilepsy, post herpetic neuralgia, diabetic peripheral neuropathy and fibromyalgia [6].

During literature search it was found that a HPLC method is reported for Quantification of Pregabalin in Human Plasma by using 1-Fluoro-2,4-dinitrobenzene as derivatization agent [7]. A UV spectroscopy method was also reported for the determination of pregabalin from bulk drug, pharmaceutical formulation and human urine [8]. Few other methods were reported for the determination of pregabalin by RP-LC [9,10]. All the reported methods are not sensitive enough
to quantify the pregabalin residue from the surfaces of equipments used in pharmaceutical manufacturing with desired accuracy and precision.

The present paper describes a validated simple and rapid reversed phase High Performance Liquid Chromatography method for estimation of pregabalin from the surfaces of equipments used for pharmaceutical manufacturing. The developed method can quantitate pregabalin accurately and precisely at residual levels from the manufacturing equipments after cleaning. Method validation was performed in accordance with International Council for Harmonization validation guidelines [11].

**Material and Methods**

**Materials and reagents**

All reagents were of analytical grade unless stated otherwise. Pregabalin working standard was supplied by IPDO, Dr Reddy's Laboratories, Hyderabad, Telangana, India. Sodium hydroxide, sodium di hydrogen phosphate monohydrate and methanol (HPLC grade) were procured from Merck, Mumbai, India. Deionized water, used for preparation of all the solutions, was procured from Merck, Mumbai. DURAPORE HVLP 0.45µm membrane filter, used for mobile phase (Buffer) filtration were procured from Millipore (Millipore, Milford, MA, USA). The swab sticks, used for extracting the drug from the stainless steel surface were procured from Texwipe (Kernersville, NC USA). The surface was selected as stainless steel, based on the material of construction of the contact part of pharmaceutical manufacturing equipment.

**Chromatographic system**

The HPLC system consisting of a G1311A quaternary pump, G1314B UV detector, a G1313A auto sampler, a G1322A degasser, a G1330B thermostat and a G1322A degasser (all from Agilent, Santa Clara, CA, 95051 United States), was used for analysis. The chromatographic and the integrated data were recorded using Empower networking software (Waters Corporation, Milford, MA, USA). The mobile phase consisting of sodium di hydrogen phosphate monohydrate buffer (pH adjusted to 6.30 with NaOH solution; 0.01M) and methanol in the ratio of 85:15 v/v respectively was filtered through DURAPURE HVLP 0.45µm membrane filter and degassed by sonication for half an hour before use. HPLC analysis was carried out on a Waters Symmetry Shield RP18 column (5.0 µm, 250 mm x 4.6 mm), manufactured by Waters Corporation, Milford, MA, USA. Column oven temperature was maintained at 40 °C and the column flow rate was kept as 0.8 mL/min. The injection volume was 500 µL. The detection was performed at 200 nm.

**Preparation of standard solution preparation**

A standard stock solution was prepared by dissolving the appropriate and accurately weighed amount of pregabalin in deionized water to obtain 0.7 mg/ml of pregabalin and then further diluted to 1.4 µg/mL.

**Preparation of test tubes and swabs**

Water was used as a swabbing solvent to remove the pregabalin residue from manufacturing surface. Required numbers of 20 mL screw cap glass test tubes, containing one Texwipe swab stick and 10 mL of swabbing solvent, were rinsed. The swab sticks were squeezed against the sides of the test tube and the swabbing solvent was discarded.

**Blank preparation**

Accurately measured 10 mL of swabbing solvent was transferred to the cleaned and dry 20 mL screw cap glass test tubes. A clean Texwipe swab stick was placed in the test tube, placed in an ultrasonic bath for 10 minutes. The swab stick was squeezed and taken out. This solution was centrifuged at 4000 RPM for 10 min and the upper clear solution was used for HPLC analysis.

**Test preparation**

Accurately measured 10 mL of swabbing solvent was transferred to the cleaned and dry 20 mL screw cap glass test tubes. A clean Texwipe swab stick was placed in the test tube. A cleaned and dried stainless steel plate (4” x 4”) was taken and 1 mL of standards solution was spiked on it. The plate was allowed for drying. After drying the swabbing was performed by covering the total surface. Swabbing was first done horizontally and then vertically, starting from outside toward the center with the swab stick moistened with the swabbing solvent. Swab stick was placed in the test tube. The test tube was placed in an ultra-sonic bath for 10 minutes for sonication. After sonication the swab stick was squeezed and taken out. This solution was centrifuged at 4000 RPM for 10 min and the upper clear solution was used for HPLC analysis.

**Calculation of limits**

The acceptance limit is generally represented as MACO (Maximum Allowable Carry Over). Different approaches can be used to calculate the MACO value, when the therapeutic daily dose is known; MACO value can be calculated by using the following formula [12].

\[
MACO = \frac{TDD_P \times BS_{min}}{SF \times MDD_N}
\]

Where TDD<sub>P</sub>: Standard therapeutic dose of previous product

BS<sub>min</sub>: Minimum batch size of the next product

MDD<sub>N</sub>: Maximum daily therapeutic dose of the next product

SF: Safety factor which is considered as 1000.

\[
SAL = \frac{MACO \times A_s \times R_f}{EQ_{1a}}
\]

Where SAL: Swab acceptance limit

MACO: Maximum allowable carryover,

A<sub>s</sub>: Sampling area from where the swab was collected,

R<sub>f</sub>: recovery factor and

EQ<sub>1a</sub>: total surface area of equipment.

The detection limit of the analytical method should be sufficiently sensitive to detect the established acceptable level of the residue.
Method Validation

The following validation parameters were performed: Specificity, recovery, Limit of Detection (LOD), Limit of Quantitation (LOQ), linearity, ruggedness and robustness.

System suitability

System suitability parameters were measured to verify the system performance. Standard solution containing 1.4 µg/ml of pregabalin was injected to establish system precision. Six replicate injections of standard preparations were injected in HPLC and the relative standard deviation (RSD) was calculated for peak area of pregabalin. USP tailing was measured for pregabalin peak from standard solution. The acceptance criteria for RSD for pregabalin peak areas and USP tailing factor were less than 10.0% and 2.0 respectively.

Specificity (Swab interference)

This test is required to establish the specific nature of the method for estimating the analyte. It was established by analyzing the swabbing solvent, swab interference sample (in duplicate), pregabalin standard solution and pregabalin test preparation, as per test method.

Recovery

The recovery study of pregabalin was performed by spiking the known amount (6.3 µg) of pregabalin on cleaned and dried stainless steel plate (4” x 4”). Swabbing was performed by Texwipe swab, as per the method with 10 ml of swabbing solvent. Samples were prepared in triplicate and injected in to HPLC. % Recovery was calculated.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Both pregabalin’s LOD and LOQ were established respectively by identifying based on a visual peak and quantitative concentration with desired precision.

Precision at limit of quantification

Six test preparations, having pregabalin at the level of about limit of quantification were prepared and injected into the system. The % RSD for six replicate preparations was calculated.

Linearity

Linearity was established by plotting a graph with concentration versus area response of pregabalin and determined the correlation coefficient. The solutions were prepared at seven concentration levels starting from about LOQ level of 0.3917 µg/ml up to 2.7979 µg/ml. Calibration curves were plotted between the analyte concentration and the peak areas. The Correlation coefficient value, slope and y-intercept were calculated.

Robustness

To establish the robustness of the developed method, experiments were performed by deliberately altering the conditions. System suitability parameters were evaluated during this study. Changes in the following variables were tested: pH of buffer in mobile phase from 6.1 to 6.5 (± 0.2 units), column temperature 35°C to 45 °C (± 5°C), column flow rate from 0.6 ml/min to 1.0 ml/min (± 25%), and change in organic phase (methanol) in mobile phase 90% to 110% (±10%). Two types of filters (Nylon and PVDF) with pores size of 0.45 µm were evaluated during filter validation. Unfiltered and filtered standard preparations were injected in to HPLC and similarity factor was calculated against unfiltered standard preparation.

Solution stability and Mobile phase stability

The solution stabilities of pregabalin standard, test preparation and mobile phase were determined by keeping them on bench top at room temperature for 48 hours. The samples were then injected at every 24 hours interval. The assay value of pregabalin was estimated against a freshly prepared standard solution and the stability of mobile phase was established by keeping it in tightly closed condition on bench top for 48 hours at room temperature. The freshly prepared standard was injected by using the stored mobile phase at every 24 hours interval.

Results and Discussion

Method development and optimization

The main objective of this study was to develop this method that can quantify pregabalin at trace levels, in manufacturing equipments after cleaning. Based on the chemical structure of pregabalin it is evident that it is a very small molecule and it does not have chromophores which are responsible for absorbance in UV/visible region. Therefore the maximum selected absorption wavelength of the pregabalin was 200 nm but at the selected wavelength also the peak response was less. So for initial method assessment purpose higher injection volume (200 µL) was chosen. Based on the MACO limit, a pregabalin solution (1.4 µg/ml) was prepared in the diluent for method development.

Pregabalin is having two pKa as 4.2 and 10.6. Pregabalin is a polar compound and is highly soluble in polar solvents. Due to this pregabalin is having very poor retention in reversed phase chromatography at lower pH. So to retain pregabalin in reversed phase chromatography, the pH of the mobile phase was selected ~6-7. Initially a buffer for mobile phase was chosen as sodium di hydrogen phosphate monohydrate buffer (pH adjusted to 6.30 with NaOH solution; 0.01M). The mobile phase was prepared by mixing sodium di hydrogen phosphate monohydrate buffer (pH adjusted to 6.30 with NaOH solution; 0.01M) and acetoniitrile in a ratio of 90:10 v/v respectively, with a flow rate of 1.0 ml/minute by using Waters Xterra column (3.5 µm, 100 mm x 4.6 mm). Column oven temperature was kept as 30°C. Pregabalin solution (1.4 µg/ml) was injected. It was observed that pregabalin was eluting in void and the peak shape was not symmetrical. So to retain pregabalin peak, organic portion in mobile phase (Acetonitrile) was replaced by methanol. Pregabalin peak was retained but the still it was eluting close to void and peak was not symmetrical, a broad peak was observed. Peak area for pregabalin peak was also less. To retain it further different columns and different ratio of mobile phase buffer were tested. pH of mobile phase was increased by 0.5 units but no significant improvement was observed. It indicated that a small change in pH does not have any significant impact on the retention time of pregabalin so pH was kept 6.30 for further experiment also. To retain pregabalin peak, column was changed to Waters Symmetry Shield RP18 column (5.0 µm, 250 mm x 4.6 mm). This column has the polar end capping so due to this it gives better retention for polar analyte. By changing the column pregabalin was separated from void but still peak response was less for pregabalin. The mobile phase ratio was optimized by testing different...
Figure 1: Representative chromatograms of Pregabalin (a) Blank preparation, (b) Standard preparation, (c) Swab blank preparation (d) Test preparation at limit of detection level and (e) Test Preparation.
ratio of buffer and methanol. The optimum retention was observed with the mobile phase consisting of sodium di hydrogen phosphate monohydrate buffer (pH adjusted to 6.30 with NaOH solution; 0.01M) and methanol in the ratio of 85:15 v/v respectively. Injection volume was optimized by injecting the pregabalin solution with different injection volumes (200 µL, 400 µL, 500 µL). Based on the peak responses and the MACO limit, injection volume was finalized as 500 µL. To increase the sensitivity column oven temperature was changed to 40°C.

Finally the mobile phase consisting of sodium di hydrogen phosphate monohydrate buffer (pH adjusted to 6.30 with NaOH solution; 0.01M) and methanol in the ratio of 85:15 v/v respectively were found suitable. Flow rate of 0.80 ml/min was finalized. The solution; 0.01M) and methanol in the ratio of 85:15 v/v respectively.

Method Validation Results

System suitability

System suitability is demonstrated to confirm the suitability of the system before starting the analysis. The system suitability was established based on RSD (%) for pregabalin peak areas from six standard replicates, and USP tailing factor for pregabalin peak from standard preparation. System suitability parameters were found within the acceptance limits (Table 1). Representative chromatogram of blank preparation and standard preparation is presented in (Figure 1a, 1b).

Specificity

The specificity of the test method was established by analyzing the swabbing solvent, swab interference sample (in duplicate), pregabalin standard solution and pregabalin test preparation, as per test method. No interference was observed at the retention time of pregabalin due to swab stick and swabbing solvent (Figure 1c).

<table>
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<tr>
<th>Parameter</th>
<th>Specification</th>
<th>Observed Value</th>
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<tbody>
<tr>
<td>USP Tailing</td>
<td>≤ 2.0</td>
<td>1.1</td>
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<tr>
<td>Area [RSD (%), n=6]</td>
<td>≤10.0</td>
<td>0.3</td>
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RSD: Relative standard deviation.

<table>
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<tr>
<th>Parameter</th>
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<tr>
<td>LOD (µg/ml)</td>
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<tr>
<td>LOQ (µg/ml)</td>
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<tr>
<td>Precision at LOQ</td>
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<tr>
<td>Coefficient correlation</td>
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<td>Intercept (a)</td>
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<td>Slope (b)</td>
<td>42048.374</td>
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<tr>
<td>Recovery (%)* at stainless steel plate</td>
<td>95.5 ± 1.3</td>
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Table 1: System suitability parameters.

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<tr>
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<th>Observed Value</th>
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<tr>
<td>Parameter</td>
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<tr>
<td>LOD (µg/ml)</td>
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<td>Recovery (%)* at stainless steel plate</td>
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</tr>
</tbody>
</table>

*Mean ± RSD (%) for three determinations.

Limit of detection and Limit of quantification

Limit of detection and limit of quantification for pregabalin were established based on visual method. The limit of detection, limit of quantification values are reported in Table 2.

Precision at limit of quantification

Precision of pregabalin at about limit of quantification was demonstrated in form of RSD [%] for six replicate preparations of pregabalin at about limit of quantification. The RSD was observed as 2.70%. It shows that method is precise (Table 2). Representative chromatogram of limit of quantification sample is presented in Figure 1d.

Linearity

Linearity was proved for pregabalin from concentration levels ranging from about limit of quantification level (0.3917 µg/mL) to 2.797 µg/mL. The correlation coefficient value was found 0.999 for pregabalin (Table 2).

Accuracy

The percentage recovery for pregabalin was found more than 93.5%. The chromatogram of recovery sample is shown in Table 3. The % recovery values for pregabalin are presented in Table 2. Representative chromatogram of accuracy sample is presented in Figure 1e.

Robustness

In all the deliberate varied chromatographic conditions (flow rate, column temperature, mobile phase pH and composition of organic solvent no significant change was observed in the retention time of pregabalin. The tailing factor for pregabalin peak was found 1.0, and RSD for peak areas was less than 1.0% (Table 3). In filter validation, similarity factor values for both the filters, NYLON and PVDF, were found in the range of 1.00 to 1.01. It shows that both the filters are suitable for filtration.

Solution stability and mobile phase stability

The variability in the estimation of pregabalin was within 2.4% from the initial value, during solution stability. The results from solution stability and mobile phase stability experiments confirmed that standard solutions, test preparations and mobile phase were stable up to 48 hours on bench top.
Conclusion

A simple and rapid reverse-phase HPLC method was developed and validated for estimation of pregabalin at trace level from the surfaces of pharmaceutical manufacturing equipments after cleaning. All the method validation parameters were found meeting the acceptance criteria. The method was found specific, precise, accurate, rugged, robust, and linear. Hence the method can be used during cleaning validation of pregabalin during manufacturing of pregabalin.

Acknowledgements

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References