Analytical RP-HPLC Method for Development and Validation of Pregabalin and Methylcobalamin in Combined Capsule Formulation

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Abstract
This work is concerned with application of simple, accurate, precise and highly selective reverse phase high performance liquid chromatographic (RP-HPLC) method for determination of pregabalin and methylcobalamin combined in capsule dosage form. Chromatographic separation was achieved isocratically using Waters alliance 2695 seperation module, C18 column (250 x 4.6 mm, 5 mcg/ml) at temperature 40°C. Flow rate selected was 1ml/min using UV visible PDA detector at 210nm. Mobile phase was prepared using ammonium dihydrogen-o-phosphate (buffer 6.0), acetonitrile and methanol in the ratio of 75:15:10 which gave better resolution and sensitivity. Developed method was validated in terms of linearity, range, specificity, precision, accuracy, robustness and ruggedness. The limit of detection was found to be 47.93mcg/ml for pregabalin and 1.33 mcg/ml for methylcobalamin respectively. The limit of quantitation was found to be 145 mcg/ml and 4.05 mcg/ml for pregabalin and methylcobalamin respectively. The method was found to be linear in the range of 3200-4800 mcg/ml and 16-24 mcg/ml for pregabalin and methylcobalamin respectively with correlation coefficients of 0.9938 and 0.9959. Content of pregabalin and methylcobalamin was found to be 99.17 and 100.15% respectively. The validation of proposed method was verified by recovery studies and was found to be satisfactory.

Key words: RP- HPLC, Pregabalin, Methylcobalamin.

Introduction
PRE [2] chemically known as (S)-2(aminomethyl)-5-methylhexanoic acid is used as anticonvulsant, neurotransmitter. It binds with high affinity and specificity to voltage-gated calcium channel alpha (2)-delta proteins [5,6]. It is freely soluble in water both in acid and basic aqueous solution. It is well absorbed after oral administration and largely excreted by renal excretion.
MCA[2] is a vitamin and chemically it is known as 1 amino propane 2-ol 15,6, dimethyl imidazole(ii) 5,6 dimethyl benzimidazole-D-ribofuranoside(iii) and 3 methyl. It is freely soluble in water.

According to the literature survey carried out, pregabalin is not official in pharmacopoeia, but methylcobalamin is official in USP, EP and BP. A simple sensitive, selective, simultaneous determination has already been developed for determination of Pregabalin, Gabapantin and Vigabatrin in human serum, and also for methylcobalamin a routine estimation in liver, plasma, milk, intestinal fluid and faeces has been reported. But there was no analytical method reported for estimation of both drugs in the combination. Hence a RP-HPLC [3,4] method was developed and validated as per ICH guidelines[8].

**Experimental**

**Materials**

UV cabinet (shimadzu), HPLC (Waters alliance 2695 separation module), column (hypersil BDS), C18 column (250 x 4.6 mm, 5mcg/ml), analytical balance (Shimadzu libror), and pH meter (control dynamics).

Acetonitrile from Ranbaxy laboratories, glacial acetic acid AR, methanol AR from S.D fine chemicals, pregabalin from Seemed lab limited, methylcobalamin from Biocon limited, Whatman GFC filter paper, ammonium dihydrogen-o-phosphate were used.

**Methods**

The HPLC [7] system was operated isocratically at flow rate of 1ml/min, 40°C. Mobile phase was prepared using ammonium dihydrogen-o-phosphate (pH 6.0), acetonitrile and methanol in the ratio of 75: 15:10 which gave better resolution and sensitivity. Detection was carried out at 210 nm.

**Preparation of standard solutions**

**Pregabalin**

About 200mg of pregabalin was weighed and transferred to class A 50ml volumetric flask containing buffer : acetonitrile : methanol in the ratio 75: 15:10. Volume was made up to 50ml.

Sufficient dilution were made to get concentration of 4000mcg/ml of pregabalin.

**Methylcobalamin**

20mg of methylcobalamin was taken and transferred to class A 100 ml volumetric flask and volume was made up with buffer : acetonitrile: methanol in the ratio 75: 15:10 as diluent. 5 ml of above solution was transferred to 50 ml volumetric flask and finally volume was made to 50
ml with the diluent to get final concentration of 20mcg/ml

**Preparation of sample solution**

20 capsules were selected randomly for analysis. 426mg of the sample was taken in 50ml volumetric flask and diluted with suitable diluent and the final volume was made up with the diluent used. Above solution was filtered through nylon millipore filter paper and 100μl of this solution was injected and then chromatogram was recorded. The amount of pregabalin and methylcobalmine were calculated by comparing standard with test.

![Sample Information for standard Pregabalin and Methylcobalmine.](image)

Above Figure 1 was the chromatogram obtained for the standard pregabalin and methylcobalmine having retention time of 4.161 and 7.804, respectively.

**Results and Discussion**

A RP-HPLC method was developed and validated as per ICH guidelines for combination of pregabalin and methylcobalmine. It was validated for linearity, range, specificity, precession, accuracy and robustness.

**Table 1. Method precession.**

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>PREGABALIN</th>
<th>METHYLCOBALAMINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avge. % label claim</td>
<td>99.77%</td>
<td>100.15%</td>
</tr>
<tr>
<td>Std deviation</td>
<td>0.36766</td>
<td>1.4251</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.36848</td>
<td>1.422</td>
</tr>
</tbody>
</table>
Table 2. Recovery studies.

<table>
<thead>
<tr>
<th>LEVELS %</th>
<th>AMOUNT RECOVERED IN mg</th>
<th>% RECOVERY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE</td>
<td>MCA</td>
</tr>
<tr>
<td>80</td>
<td>137.2</td>
<td>0.667</td>
</tr>
<tr>
<td>100</td>
<td>166.4</td>
<td>0.834</td>
</tr>
<tr>
<td>120</td>
<td>196.2</td>
<td>0.974</td>
</tr>
</tbody>
</table>

The retention time of pregabalin and methylcobalamine was found to be 4.168 and 7.804 respectively. The method precession for pregabalin and methylcobalamine given in Table 1 was found to be 99.77% and 100.15% respectively. The tailing factor of pregabalin and methylcobalamine was 0.9661 and 1.5405 which indicates symmetrical nature of the peak. System suitability parameter were studied and found to be satisfactory. The resolution was found to be 7.1990. Number of theoretical plate was 2119.045 and 2559.81 which indicates efficient column performance. The retention time was found to be within limit of 0-10 min. This parameter indicates the specificity of the method. The linearity studies were performed and were found to be linear in the range of 3200 to 4800 mcg/ml for pregabalin and 16 to 24 mcg/ml for methylcobalamine for target concentration. The recovery studies given in Table 2 satisfied the percentage recovery range. The proposed method was verified by system precision and method precession. The %RSD for system precession of pregabalin was 0.4762 and for methylcobalamine was 1.346.

Conclusion
The proposed method was found to be simple, sensitive, rapid and economical for determination of pregabalin and methylcobalamine in the combined drug formulation. The sample recovery in all formulation was in good agreement with their respective label claims and they suggested non interference of formulation excipients in the estimation. Hence it can be easily and conveniently adopted for routine analysis.

References


[7] Therapeutic drug monitoring, ISSN 0163-4356 CODEN TDMODV.