Carisoprodol is a muscle relaxant drug. In this study of drug product of carisoprodol, two impurities (referred as impurity-B and impurity-D) were detected at levels of 0.5% and 0.6% by gradient reverse phase HPLC method. It is pharmacopeia product but there is no high performance liquid chromatography method in any pharmacopeia, hence, it is necessary to develop a RP-HPLC method to identify the impurities. Carisoprodol does not have chromophoric group. Hence, a novel method was developed by using zorbax eclipse XDB-C8 250 X 4.6 mm. The purities of two impurities were undertaken using the structural studies of the impurities using NMR. Based on the spectral data the structures of impurity-B and impurity-D have been confirmed respectively. A detailed method development and validation study of carisoprodol and its impurities are presented in this article.
2.1 INTRODUCTION

Carisoprodol is a centrally acting muscle relaxant with analgesic properties, making a popular drug of abuse. Carisoprodol is a dicarbomate, centrally acting, oral skeletal muscle relaxant whose chief application is in the treatment of acute muscular spasm associated with craniomandibular disorder, lumbago, sciatica and other lower back syndromes [1]. Carisoprodol chemical name being N-isopropyl-2-methyl-2-propyl-1, 3-propanediol dicarbamate, is a pharmaceutical active agent whose metabolite is meprobamate (impurity-D) [2]. Carisoprodol, a synthetic compound first synthesized in 1959 is related structurally to meprobamate. It is prescribed on its own, or in combination products containing phenacetin, caffeine and codeine [3, 4]. Carisoprodol is widely used in primary care setting for the treatment of musculoskeletal conditions associated with muscle spasms and back pain. After its introduction, a number of reports have suggested that the drug may have a potential for abuse, there are many reports of the development of abuse and dependence involving carisoprodol and meprobamate [5-11]. However, carisoprodol is not a controlled substance at the federal level. The diversion and abuse of carisoprodol and its adverse health effects have substantially increased over the last several years. According to the Drug Abuse Warning Network, the number of emergency department episodes involving carisoprodol were 6569 in 1994, 7771 in 1995, 11,239 in 2001, 10,094 in 2002, 17,366 in 2004, and 19,513 in 2005 [10, 11]. These figures represented an almost 300% increase from 1994 to 2005. According to data from the National Survey on Drug use and Health from 2002–2005, the occurrence of misuse of carisoprodol was approximately equal to that of clonazepam [12]. The effect of carisoprodol, which includes sedation, loss of balance, confusion and increased
reaction time are similar to those of alcohol, benzodiazepines and other CNS depressants and well documented to decrease human performance and adversely affect driving effect [13]. The structure of carisoprodol is such that it does not have UV chromophore with significant absorbance. Therefore, the USP assay method for carisoprodol tablets employs a liquid chromatography equipped with a refractive index detector [14]. Official monographs available for carisoprodol drug substance refers to TLC method for impurity estimation [15]. Several methods were reported based on titrimetric, infrared, nuclear magnetic resonance (NMR), gas chromatography, liquid chromatography (LC) and gas chromatography-mass spectrometry (GC-MS) for the determination of carisoprodol analysis [16-27]. The current study describes the UV-LC method for determination of carisoprodol and its impurities. The method was developed and validated as per ICH guidelines. The chemical structures are as shown in figure from 2.1a to 2.1c.

Fig 2.1a. (2RS)-2-[(Carbamoyloxy) methyl]-2-Methylpentyl (1-methyl ethyl) carbamate (Carisoprodol).

Fig 2.1b. 2-methyl-2-propylpropane-1, 3-diyl dicarbamate (Impurity-D)
Fig 2.1c. N-isopropyl-2-methyl-2-propyl-3-hydroxy propyl carbamate (Impurity-B)

2.2 EXPERIMENTAL

2.2.1 Chemical and reagents

HPLC grade acetonitrile was purchased from Merck, water used was from a milli-Q purified system, Millipore, orthophosphoric acid from Merck and triethyl amine was purchased from spectrochem. Carisoprodol, impurity-D and impurity-B were obtained from chemical research and development department, Troy Life sciences, Bangalore. Chloroform-d3, methanol-d4 and dimethyl sulphoxide-d6 (for NMR) were purchased from Aldrich chemical co. USA.

2.2.2 High performance Liquid Chromatographic conditions

Samples were analyzed on Shimadzu prominence separate module LC-10AD equipped with PDA detector Empower-2 software was used for data acquisition and processing. Zorbax eclipse-XDB C (8) HPLC column was maintained at 30 ± 2°C. The buffer preparation was by dissolving 5.5ml of orthophosphoric acid in 950 ml of water, pH adjusted to 3.1 by using triethyl amine. The mobile phase pump-A was mixture of buffer and acetonitrile in the ratio (67:33 v/v) and the mobile phase pump-B was mixture of buffer and acetonitrile in the ratio (33:67 v/v). The flow rate was
set at 1.0 ml/min and UV detector at 200 nm. The injection volume was 20 µl. The
gradient elution was (Tmin A: B) T0 100:0, T25 100:0, T30 70:30, T40 50:50, T45
100:0. The diluent used was mobile phase-A throughout the analysis.

2.2.3 Preparation of stock solution for method validation
A test preparation of 9 mg/ml of carisoprodol API sample was prepared by dissolving
in diluent (mobile phase-A). A stock solution of impurity-D was prepared by
dissolving 90.09 mg of standard into 100 ml volumetric flask and made up to the
volume with the diluent. 5 ml of above solution was transferred into a 100 ml
volumetric flask and made up to the volume with the diluent. The standard solution of
impurity-D was prepared at 0.5% with respect to sample concentration (9 mg/ml). A
stock solution of impurity-B was prepared by dissolving 90.06 mg of standard into
100 ml volumetric flask and made up to the volume with diluent. 2 ml of above solution
was transferred into a 100 ml volumetric flask and made up to the volume with the
diluent. The standard solution of impurity-B was prepared at 0.2% with respect to
sample concentration (9 mg/ml).

2.3 NMR spectroscopy
$^1$H NMR spectra were recorded at 400 MHz, using Bruker 400MHZ spectrometer
(Bruker, Falladen, Switzerland) equipped with a 5 mm BBO probe and Z-gradient
shim system. The $^1$H spectra were recorded with 1s pulse repetition time using 30°
flip angle. Samples were dissolved in deteriorated chloroform. The $^1$H chemical shift
values were reported on the δ scale in ppm relative to TMS. All spectra were recorded
with sample spinning. The NMR spectra of impurity-B are shown in figure 2.3d, and
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the NMR spectra of impurity-D are shown in figure 2.3e. The comparative study of NMR shown in table-1.

Fig 2.3d. $^1$H NMR of impurity-B
Table 1. ¹H NMR spectral data of impurity-B and impurity-D

<table>
<thead>
<tr>
<th>Compound</th>
<th>Solvent</th>
<th>¹H NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impurity-D</td>
<td>CDCl₃</td>
<td>d 3.90 (s, 3H ), 1.20 (t, 3H ), 4.7 (NH₂)₂,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impurity-B</td>
<td>CDCl₃</td>
<td>d 4.73-4.76 (s, 1H broad), 3.79 (s, 1H OH), 3.9(s, 3H, CH₃), 0.89-1.29 [m, 10H, (CH₃)₂ (CH₂)]</td>
</tr>
</tbody>
</table>

2.4 RESULTS AND DISCUSSION

Carisoprodol lacks any appreciable UV absorbance or fluorescence, therefore, GC-MS methods are mainly used. However, problems encountered with GC are due to the
heat instability of carisoprodol at the injection port, leading to thermal decomposition which results in poor chromatography. Hence, it was necessary to developed a rugged method of UV-HPLC in order to overcome such problems. Various methods with stationary phases; diluents were used for the development of sensitive and accurate method for carisoprodol and its impurities. HPLC with UV detection was chosen as simple, fast and effective separation method for determination of Carisoprodol and its process related impurities. All compounds were tested at different wavelength and had a low detector response for detection of the compounds, however, 200nm was chosen especially with regard to absorption spectra of Carisoprodol and its impurities, both gave higher detector response at 200 nm, therefore, the final absorption wavelength for detection was chosen at 200 nm. Several analytical columns were chosen and tested during the development of this method. GL-Science inertsil ODS column, waters X-Terra column, Agilent Zorbax reversed-phase bonded phases based on ultra-pure silica-Stable bond (SB)-provided poor peak shape and sample resolution, as well as no long column lifetimes. Different mobile phases consisting of acetate buffer, formate buffer, mixture of acetonitrile and water were tested with different pH. Numerous trials were carried out with different sample preparation solutions to enhance peak responses. For instance methanol, acetonitrile, mixture of acetonitrile and water also was used. Even derivatizations of samples were carried out using picrate derivative and benzoyl derivative. Sample solutions were also prepared by mixing methanol: perchloric acid: triethyl amine and acetonitrile: perchloric acid: triethylamine. Also with above mentioned combinations, the solution was heated at 30°C for few minutes. Even after so many experiments it was hard to optimize the HPLC method. Finally, the best results were obtained for the analysis using a buffer consisting of 5.5ml phosphoric acid in 950 ml of water, pH adjusted to 3.1 by using
triethyl amine and the HPLC Zorbax eclipse XDB C (8) column (250 X 4.6mm 5um). The mobile phase-A consisted mixture of buffer and acetonitrile in the ratio (67:33 v/v) and the mobile phase-B consisted mixture of buffer and acetonitrile in the ratio (33:67 v/v). Ultimately, the sample was prepared by using mobile phase-A. Thus, a significant HPLC method was developed and optimized. In this method Impurity-B RRT was about 2.29 and impurity-D RRT was about 0.31. A thin layer chromatography of Carisoprodol as per USP pharmacopeia is also shown in figure 2.4f. A typical impurity spiked chromatogram is depicted in figure 2.4g.

Fig 2.4f. Thin layer chromatography of Carisoprodol and impurity-D
2.5 METHOD VALIDATION

A newly developed and optimized method was validated for quantitation limit (QL), detection limit (DL), linearity, precision, accuracy, specificity and robustness as per ICH guidelines. Validation was carried out for two process related impurities, viz. impurity-B and impurity-D. Validation study was carried out for impurity-B and impurity-D. The selectivity was checked by injecting 9mg/ml of Carisoprodol solution containing 0.2% of impurity-B and 0.5% of impurity-D monitored throughout the validation. Method validation results are summarized in table-2.

2.5.1 Specificity

To demonstrate the specificity of HPLC method for all the impurity spiked samples, the purity angle obtained for Carisoprodol and impurity peaks was less than purity threshold demonstrating spectral homogeneity. During this study impurity-B and
impurity-D were well separated from each other and as well as from Carisoprodol which proved that the adopted method was specific.

2.5.2 Linearity, RRF, Detection limit (DL) and Quantitation limit (QL)

The linearity was established by measuring area responses for impurity-B and impurity-D, linearity ranging from QL to 200% with respect to sample concentration (9mg/ml). Seven concentrations were prepared across the range and injected in triplicates. The average area calculated was plotted against the concentration. The correlation co-efficient obtained was greater than 0.99 for impurity-B and impurity-D. The results are presented in table-2. The quantitation limit (QL) and detection limit (DL) for Carisoprodol impurities were determined by signal to noise ratio method. The typical QL chromatogram of impurities is shown in figure 2.5.2h. The linearity curves for impurity-B and impurity-D is shown in figure 2.5.2i and 2.5.2j.

![Fig 2.5.2h. LOQ chromatogram of impurity-D and impurity-B](image-url)
2.5.3 Precision and accuracy

The precision of the related substance method was checked by injecting six individual preparations of (9 mg/ml) Carisoprodol spiked with 0.2% of impurity-B and 0.5% of impurity-D. Percentage RSD for peak areas of each impurity was calculated and study was also performed in the same procedure on a different day. The intermediate precision of the method was also evaluated by a different analyst and different instrument in the same laboratory. Percentage RSD of areas of each impurity was less
than 5.0, confirming good precision. Accuracy was validated through recovery experiments by spiking known amount of impurity (50%, 75%, 100%, 125% and 150%) with Carisoprodol with respect to sample concentration (9mg/ml). Each parameter was analyzed in triplicates and percent recoveries were calculated. The results of accuracy and precision are shown in table-2.

### 2.5.4 Solution stability and mobile phase stability

Required analysis was carried out regarding solution stability and mobile phase stability. No significant changes were observed in both the solutions, also it was found to be stable up to 48 hours.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Impurity-B</th>
<th>Impurity-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method Precision (RSD)</td>
<td>1.21</td>
<td>0.56</td>
</tr>
<tr>
<td>RT</td>
<td>37.586</td>
<td>5.249</td>
</tr>
<tr>
<td>RRT</td>
<td>2.23</td>
<td>0.31</td>
</tr>
<tr>
<td>RF</td>
<td>0.50</td>
<td>9.31</td>
</tr>
<tr>
<td>Linearity ($r^2$)</td>
<td>0.998</td>
<td>0.997</td>
</tr>
<tr>
<td>Quantitation limit (%)</td>
<td>0.015</td>
<td>0.26</td>
</tr>
<tr>
<td>Detection limit (%)</td>
<td>0.008</td>
<td>0.13</td>
</tr>
<tr>
<td>Precision at QL (RSD)</td>
<td>3.56</td>
<td>2.86</td>
</tr>
<tr>
<td>% Recovery at QL (n=3)</td>
<td>93.5</td>
<td>98.6</td>
</tr>
<tr>
<td>Accuracy (% Recovery)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50%</td>
<td>92.01</td>
<td>102.94</td>
</tr>
<tr>
<td>75%</td>
<td>95.81</td>
<td>100.05</td>
</tr>
<tr>
<td>100%</td>
<td>97.11</td>
<td>99.40</td>
</tr>
<tr>
<td>125%</td>
<td>102.61</td>
<td>104.12</td>
</tr>
<tr>
<td>150%</td>
<td>108.06</td>
<td>107.43</td>
</tr>
</tbody>
</table>

n-number of determinations, RT- retention time, RRT- relative retention time, $r^2$: correlation coefficient, RF- Response factor

**Table 2. Method validation summary results**
2.6 CONCLUSION

The newly developed UV-HPLC method suggested was found to be simple, accurate, selective and equally sensitive. The method was fully validated showing satisfactory data for all the method validation parameters tested. The developed method can be conveniently used by quality control department to determine the related substances in regular Carisoprodol production samples.
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2.7 REFERENCES


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