

# Analytical Method Development and Validation of Carisoprodol in Bulk Drug and Formulation by RP-HPLC Method

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## ABSTRACT

To determine Carisoprodol in bulk and pharmaceutical formulation an accurate, simple, precise rapid and reproducible reverse phase HPLC method was developed and validated. The quantification was accomplished using Genesis C<sub>18</sub> (100x4.5mm; 4µm in particle size) column run in isocratic mode using mobile phase comprising of Acetonitrile and 1.00% triethylamine (TEA) in water, pH altered to 3.50 with orthophosphoric acid (10% v/v) in ratio of 35:65 v/v and a wavelength of 194nm was used for detection. 50.00µl was injected at 1.00ml/min(flow rate). The retention time (RT) of Carisoprodol was established at 6.96±0.12 (n=6). The method was validated in terms of linearity, accuracy precision, robustness, LOQ and LOD(ICH guidelines). 5.00µg/ml to 500.00µg/mL was the linearity range of the present method with a correlation coefficient of  $r^2 = 0.9968$ . An average recovery of 100.65±0.11% was achieved. The LOQ and LOD was 1.26µg/mL and 0.41µg/mL respectively. The method was found to be applicable for assay, stability studies and dissolution of marketed Carisoprodol tablet formulation. Tablet assay using the present method was 100.72±0.63%. The % RSD from reproducibility was 0.43%. The present method is applicable for routine quality control analysis (QC) of Carisoprodol in bulk and in pharmaceutical formulations.

**KEY WORDS:** Carisoprodol, RP-HPLC, Genesis C<sub>18</sub>, Validation.

## 1. INTRODUCTION

Stability indicating assay method is a method of quantitative analysis based on the chemical and structural properties of the active ingredient present in the drug product and which will be able to differentiate each ingredient from its degradation product so that the content of the active ingredient can be measured. It can discriminate the intact drug from its degradation product(s) during the stability evaluation period under defined storage conditions. It must be very sensitive to detect and quantitate one or more degradation products.

In the structure of Carisoprodol there are less functional groups which contribute for significant Ultraviolet (UV) absorption in the 200-400nm region. The two amide moieties in the structure of Carisoprodol are the reason for UV absorption. There are  $n-\pi^*$  and  $\pi-\pi^*$  transitions in the carbonyl groups of the amide functional groups of the molecule and these transitions are the reason for UV absorption of the molecule at 194nm.

From the exclusive literature survey, it is found that very few analytical methods reported for Carisoprodol either individually or in combination with other drugs using refractive index detector, mass detector and UV detector with precolumn derivatization, but there was no stability- indicating assay method by RP-HPLC with UV detection.

In USP monograph, for the assay of carisoprodol tablets liquid chromatograph equipped with refractive index detector was indicated. Refractive index detector is not a commonly used detector in the routine quality control (QC) labs and it takes a long time to stabilize and fluctuation in lab temperature affects the stability of the detector. Reproducibility among the results is lesser than other detectors like UV and PDA detectors.

Hence a simple method was planned UV/PDA detector by utilizing the 194 nm as a  $\lambda$ -max of the drug.

**Drug profile:** Carisoprodol is chemically 2-[[[aminocarbonyl]oxy]methyl]-2-methylpentyl isopropyl carbamate and its chemical formula is C<sub>12</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>. Carisoprodol is a skeletal muscle relaxant which acts centrally and is indicated for discomfort associated with acute, painful musculoskeletal conditions in adults.

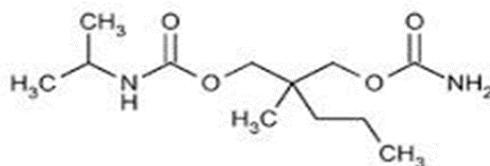


Figure.1. Chemical structure of Carisoprodol

## 2. MATERIALS AND METHODS

**Materials:** Acetonitrile, potassium dihydrogen phosphate, potassium hydroxide, sodium hydroxide, orthophosphoric acid, concentrated hydrochloric acid, hydrogen peroxide (30% v/v) were procured from Merck India Ltd, methanol HPLC grade was obtained from Finar reagents, Ahmedabad. The carisoprodol tablet was purchased from a local

pharmacy. HPLC work was achieved out using Shimadzu HPLC controlled by LC solutions software version 1.2 and provided with LC-10 ADVP pump, SIL-10 ADVP pump, Column Oven CTO-10ASVP and SPD M – 10 AVP. Separation and quantitation was done using reverse phase Genesis C<sub>18</sub> column (100x4.5mm; 4µm particle size).

#### Methods:

##### HPLC Method:

**Chromatographic conditions:** Acetonitrile and 1.00% triethylamine (TEA) in water, pH altered to 3.50 with orthophosphoric acid (10% v/v) in ratio of 35:65 v/v was taken as the mobile phase. An isocratic flow rate of 1.00mL/min was maintained while injecting the sample. A wavelength of 194nm was used for detection using UV detector with an injection volume of 50.00µL. The total runtime for analysis was kept at 10.00min.

**Standard preparation:** 10.00mg of carisoprodol reference standard was weighed in 10.0mL volumetric flask and solubilised in 5.0 mL of acetonitrile. It was sonicated for one minute and diluted up to 10.00mL with acetonitrile to obtain a 1000.00µg/ml. 100.00 µL of the above solution was taken and diluted upto 1000.00µL with the (MP) mobile phase that is Acetonitrile and Buffer (35:65) to obtain a concentration of 100.00 µg/ml. the peak area was calculated by injecting the above solution in the HPLC.

##### Assay of Formulation:

**Sample preparation:** Weigh twenty Carisoprodol tablets each containing 175.00mg of Carisoprodol were taken and their average weight was calculated, then tablets were crushed to a powder and weigh the powder equivalent to two tablets and then transfer it to a volumetric flask (100 mL), add 40 mL of acetonitrile and sonicated for 15min, cool and then dilute upto 100.00mL with Acetonitrile (3500.00µg/mL). The solution was then centrifuged at 1800rpm for 15min. The supernatant was collected and 714.00µL of the supernatant was taken and diluted upto 10.00ml with mobile phase (249.90µg/mL). This solution was then injected into the HPLC. Peak area was calculated from the recorded chromatograms for both test (tablet) and standard solution.

##### Stress testing:

##### Sample preparation:

**Acid degradation:** 10.00mg of the Carisoprodol (working standard) was weighed and emptied to volumetric flask (10.00mL) containing 8.0 mL of 1N HCl, and solubilised upto the mark and maintained at room temperature. The concentration was 1000.00µg/mL. Samples were withdrawn initially and thereafter at regular time intervals and then neutralized to 7.00 using 1N NaOH and the final dilution loaded into the HPLC system were done with the mobile phase. The standard solution of Carisoprodol in Acetonitrile was prepared which was considered as 100% for degradation study.

**Base degradation:** 10.00mg of the Carisoprodol (working standard) was weighed and emptied to volumetric flask (10.00mL) containing 8.0 mL of 1N NaOH, and solubilised upto the mark and maintained at room temperature. The concentration was 1000.00µg/mL. Samples were withdrawn initially and thereafter at regular time intervals and then neutralized to 7.00 using 1N HCl and the final dilution loaded into the HPLC system were done with the mobile phase. The standard solution of Carisoprodol in Acetonitrile was prepared which was considered as 100% for degradation study.

**Oxidative degradation:** 10.00mg of the Carisoprodol (working standard) was weighed and emptied to volumetric flask (10.00mL) containing 8.0 mL of 15% w/v H<sub>2</sub>O<sub>2</sub> and solubilised upto the mark and maintained at room temperature. The concentration was 1000.00µg/mL. Samples were withdrawn initially and thereafter at regular time intervals and the final dilution loaded into the HPLC system were done with the mobile phase. The standard solution of Carisoprodol in Acetonitrile was prepared which was considered as 100% for degradation study.

##### Dissolution:

**Sample preparation:** Six Carisoma<sup>®</sup> tablets, each containing 175.00mg of Carisoprodol were taken for dissolution. The dissolution medium was 0.05M Phosphate buffer, pH altered to 6.90 with 1N Sodium hydroxide. USP type – II apparatus (paddle) was taken for dissolution of the tablets at a speed of 75.00rpm and maintained at 37.0°C. The dissolution time was 60min within which 5.00mL samples were withdrawn at 5, 10, 15, 30, 45, 60min and replenished with buffer so as to maintain the sink conditions. The collected 5.00mL samples were injected in the HPLC after diluting to get 100.00 µg/mL concentrations.

### 3. RESULTS AND DISCUSSION

**HPLC Method development:** Because of the simpleness and ruggedness and also the lipophilic nature of Carisoprodol reverse phase HPLC was used for initial separation. Acetonitrile was selected as organic mobile phase because in acetonitrile good peak shape and peak area were observed. Moreover acetonitrile shows good UV absorbance, gives low back pressure and also has low viscosity. For method development buffers like phosphate buffer (KH<sub>2</sub>PO<sub>4</sub>) and 1% triethylamine in water were tried. Phosphate buffer was tried at pH 3.50, where 1% w/v potassium hydroxide solution was used to adjust the pH. But interferences were observed in the form of mobile phase peaks. In case of 1% triethylamine in water pH-3.50 adjusted with 10% v/v orthophosphoric acid interferences were

less and good peak shape was observed. Hence 1% Triethylamine in water pH-3.50 adjusted with 10% v/v orthophosphoric acid: acetonitrile (ACN) in the ratio of 65:35 was taken as the mobile phase.

#### HPLC Method Validation:

**Specificity:** Specificity was demonstrated using degradation studies. Carisoprodol was exposed to acid, alkali and peroxide degradation. Under all degradation conditions the peak purity of Carisoprodol passed. And there was no interference of the diluent at the retention time (RT) of Carisoprodol.

**Linearity:** The linearity curve was prepared by plotting the concentration versus area. A series of Carisoprodol standard solution were prepared over a range of 5.00 to 500.00 µg/ml over 7 points. Regression ( $r^2$ ) of 0.9968 was obtained (figure.2).

**Precision:** The repeatability precision of the present method was obtained with a %R.S.D of 0.43. The intermediate precision (inter-day) of the present method was obtained with a %R.S.D of 1.20. The results of the present method show that it is precise and reproducible (table.2).

**Accuracy:** The accuracy was performed by recovery studies. Pre analyzed standard samples were used to spike a predetermined amount of standard drug from which the recovery was calculated. Accuracy was determined at 3 levels of 120, 100 and 80% of standard concentration. Four solutions were prepared containing respectively 100.00 µg/mL standard solution, second containing 100.00 µg/mL of standard (std) spiked with 80.00 µg/mL of standard, third containing 100.00 µg/mL of standard (std) spiked with 100.00 µg/mL of standard and fourth containing 100.00 µg/mL of standard (std) spiked with 120.00 µg/mL of standard. The recoveries at three different concentrations were in the range according to ICH guidelines. Mean % recovery was  $100.65 \pm 0.11$  (table.3).

**Robustness:** Robustness evaluated incorporating small changes in the analytical method (variations in flow rate, mobile phase composition, pH of buffer) and also by observing for 24 hours the drugs stability at room temperature with diluent. The overall percentage relative standard deviation (%RSD) in the various changed parameters ranged from 0.08% to 0.96%. The results proved robustness of the method (table.4).

**Limit of Detection (LOD) and Limit of Quantitation (LOQ):** LOQ and LOD were calculated according to ICH Q2R1 guideline. LOQ and LOD were determined using S/N ratio approach. For proposed method LOQ and LOD was found to be 1.26 µg/mL and 0.41 µg/mL respectively (table.2).

**Assay of formulation:** Carisoprodol containing tablets were subjected to the analysis by the proposed method. The assay of the carisoprodol tablets was  $100.72 \pm 0.63\%$  ( $n=6$ ). This results clearly indicate that the present method can be used for the routine quality control (QC) of Carisoprodol tablets without interference from the excipients and degradation products (table.5 and figure.7).

**Stress testing:** Carisoprodol degraded under basic and peroxide conditions as evident from the decrease in peak area (table.6 and figures.2 & 3).

**Dissolution:** The limits for drug release were not less than  $80 \pm 5\%$  (Q-value) of the labeled amount of Carisoprodol in 60.00 min. The percent drug release pattern of Carisoma<sup>®</sup> tablets was concordant with the official limits of Q-value as mentioned in the USP (figures.8 and 9).

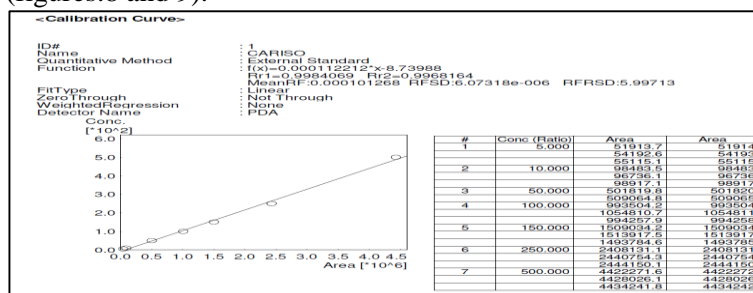


Figure.2. Calibration curve of Carisoprodol by HPLC

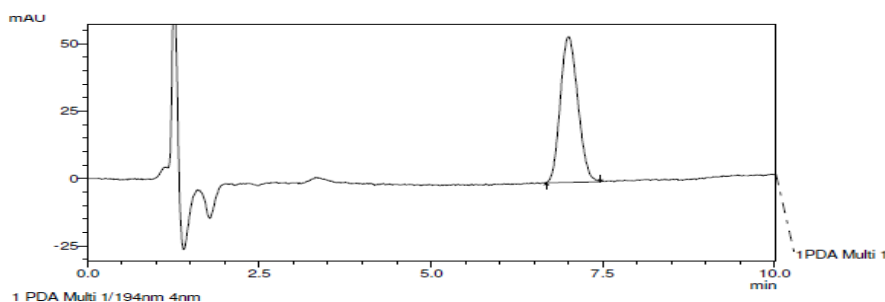


Figure.3. Representative chromatogram of 100.00 µg/mL of standard solution of Carisoprodol

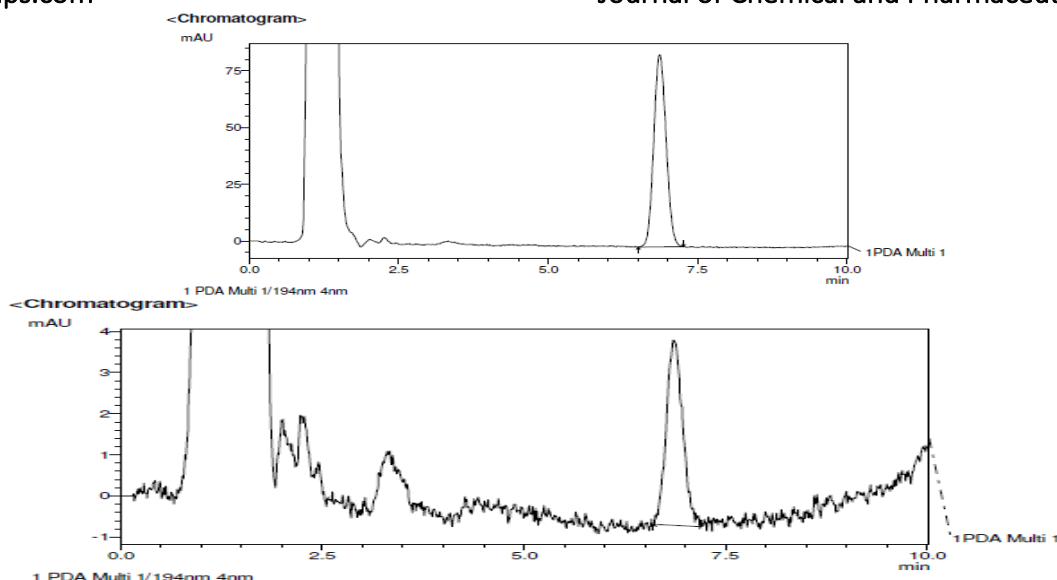


Figure.4. Chromatogram and peak purity curve for Carisoprodol in 1N HCl at room temperature (8hr)

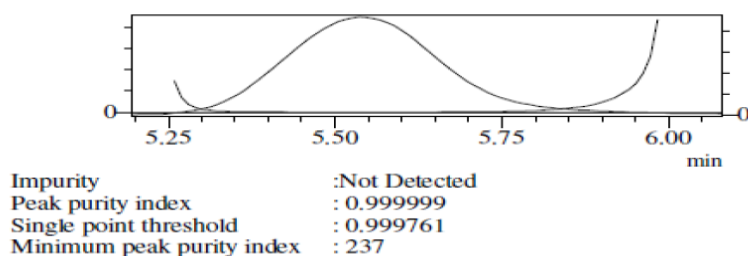


Figure.5. Chromatogram and peak purity curve for Carisoprodol in 1N NaOH at room temperature (8hr)

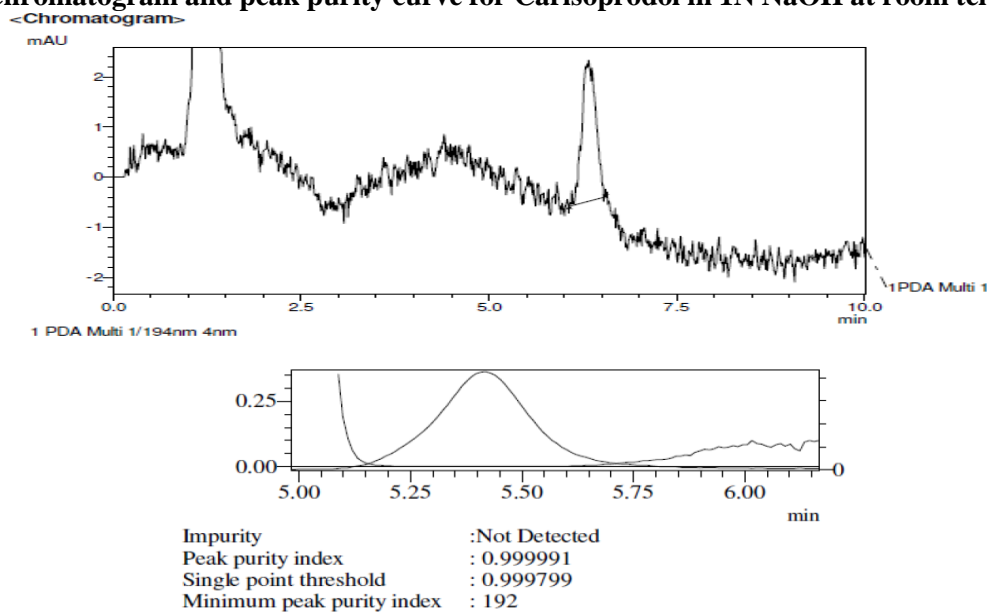


Figure.6. Chromatogram and peak purity curve for Carisoprodol in 15% H<sub>2</sub>O<sub>2</sub> at room temperature (8hr)

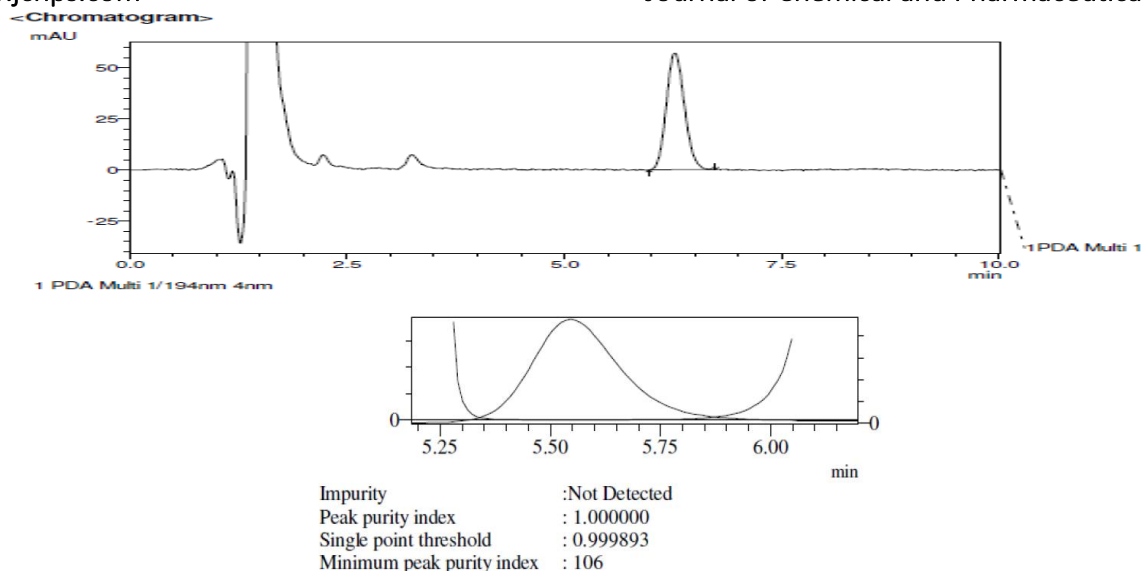


Figure.7. Chromatogram of 250.00µg/mL tablet (test) solution

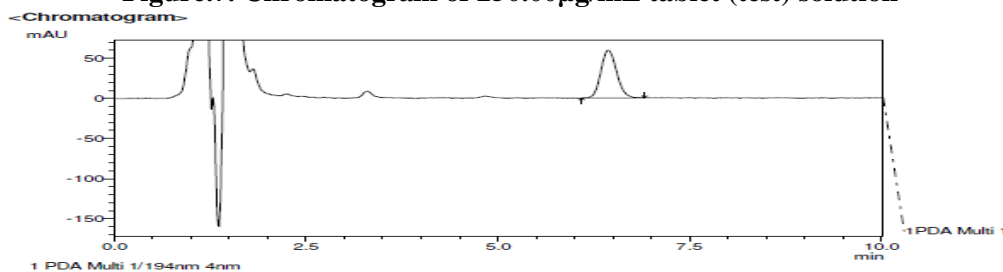


Figure.8. Representative chromatogram of marketed Carisoprodol tablets showing drug release at 60min.

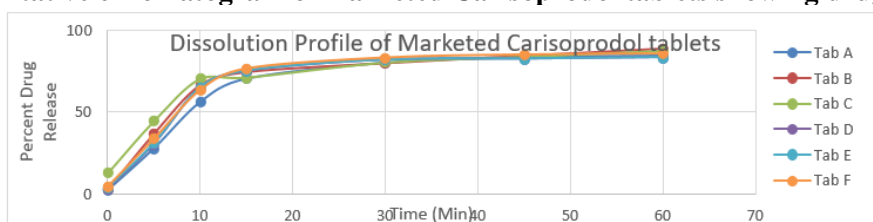


Figure.9. Dissolution profile of Marketed Carisoprodol tablets

Table.1. System suitability parameters by HPLC

Parameter	Observed Value	Acceptance criteria
Tailing factor	1.18	NMT 2.0
No. of theoretical plates	4617	NLT2000
% RSD of area	0.24	NMT 2.0%
% RSD of retention time	0.54	NMT 2.0%

Table.2. Precision, Solution stability, Linearity (n=7) and Sensitivity by HPLC

Parameter	%RSD	Limit
Precision	Repeatability	0.43%
	Intermediate	1.20%
Solution stability 10hr	0.48%	NMT 3%
Linearity (r <sup>2</sup> )	0.9968	NLT 0.9900
LOD	0.41µg/mL	--
LOQ	1.26µg/mL	--

Table.3. Accuracy by HPLC

Amount added	% Mean recovery±SD
80.00µg/mL	100.76±0.53
100.00µg/mL	100.54±0.06
120.00µg/mL	100.64±0.25
Average recovery	100.65±0.11

**Table.4. Method robustness by HPLC**

Parameter Changed		Area response (average, n=6)	%RSD
Flow rate (mL/min)	0.90	940004	0.18
	1.10	943634.80	0.12
pH	3.30	945642.50	0.10
	3.70	935089.50	0.08
Mobile phase ratio	63:37	951954.34	0.64
	67:33	963171.00	0.96

**Table.5. Formulation assay by HPLC**

Formulation	Label Claim	% Assay±SD (n=6)
Marketed Tablet	Each tablet contains 175mg of Carisoprodol	100.72±0.63

**Table.6. Forced degradation studies of Carisoprodol**

Name	Condition	% degradation	Peak Purity
Acid Degradation	1.00M HCl at room temperature for 8 hour	No Degradation	Passes
Base Degradation	1.00M NaOH at room temperature for 8 hour	32.68	Passes
Oxidative Degradation	15.00% w/v H <sub>2</sub> O <sub>2</sub> at room temperature for 8 hour	35.12	Passes

#### 4. CONCLUSION

In this HPLC method, detection of analyte at a  $\lambda_{max}$  which is below the actual UV absorption region of 200 – 400nm is particularly very critical because at these lower wavelength regions solvent cut off wavelengths comes into picture. As a result there is chance of peaks arising due to solvents. But this problem did not arise because of the strong UV absorption by the amide functional groups in Carisoprodol at 194nm.

The proposed RP-HPLC (UV detector) was simple, rapid, accurate and precise for estimation of Carisoprodol in bulk as well as for tablet formulation and available method mainly used derivatization(pre-column) technique using UV detector which itself very tedious and time consuming and Refractive index detector and mass detector which is very uncommon in the routine quality control(QC) lab. Based on these evidence it can be stated that the method is highly useful, simple, economical and it is recommended for routine use in QC laboratories and stability studies.

#### 5. ACKNOWLEDGEMENTS

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