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Original Article

# STABILITY INDICATING RP-LC ASSAY METHOD FOR CARISOPRODOL

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## ABSTRACT

**Objective:** A reverse phase stability-indicating HPLC method was developed for the determination of Carisoprodol in pharmaceutical dosage forms. The chromatographic elution was achieved on C18, 250 mm × 4.6 mm, 5-µm particle size column.

**Methods:** The mobile phase contains a mixture of water and acetonitrile in ratio of 60:40 v/v. The flow rate was 1.0 ml $min^{-1}$ and was detected by Refractive index detector.

**Results:** The method was proven to be linear over a range of 1 to 4 mg/ml with a mean correlation coefficient of 0.99998. The %mean recovery is in the range of 100.55% to 101.11% and %RSD was less than 1.0% between preparations. The % RSD for Assay results of initial sample preparation in different intervals of 0hr, 24 h, 30 h and 48 h was less than 1.0%. To establish stability-indicating capability of the method, drug product was subjected to the stress conditions of acid, base, oxidative, hydrolytic, thermal and photolytic degradation. The degradation products were well resolved from Carisoprodol.

**Conclusion:** The developed method was validated as per international ICH guidelines with respect to specificity, linearity, accuracy, precision and robustness.

**Keywords:** Carisoprodol, Stability-Indicating HPLC Method, Stress Conditions

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## INTRODUCTION

Carisoprodol is chemically (1-Methylethyl) carbamic acid 2-(((amino-carbonyl)oxy)methyl)-2-methyl pentyl ester marketed under the brand name soma since 1959 and is used as skeletal muscle relaxant [1-2]. It belongs to the class of carbamates and produces effecs associated with barbitutates [3-4]. Mechanism of the activity of carisoprodol of relieving discomfort associated with acute painful musculoskeletal condition has not been clearly known. In animal studies, muscle relaxation induced by carisoprodol was found to be associated with altered inter-neuronal activity in the spinal cord and in the descending reticular formation of the brain, resulting in blocking pain sensations between the nerves and the brain [4].

Literature review reveals few reports for carisoprodol assay. They include liquid chromatography-tandem mass spectrometry [5-6], gas chromatography [7-8], high-performance thin-layer chromatography [9] and homogeneous immunoassay [10]. All these analytical techniques have been employed for carisoprodol determination in biological samples such as urine and serum of equine and urine and plasma of human. Furthermore the reported methods are cumbersome and require sophisticated equipment. Drugs in bulk and pharmaceutical dosage forms can be analysed in quality control laboratories and cost effective methods like uv/visible spectroscopic method or HPLC with UV/Visible detector. Three extractive spectrophotometric methods for the quantification of carisoprodol in pure and pharmaceutical formulations were determined by Ravi *et al.* [11], which were based on formation of colored chloroform extractable ion-pair complexes with dyes like bromocresol green, bromothymol blue and bromophenol blue in acidic medium. However, these methods suffer from one or the other disadvantage such as extraction of ionpair complex, poor sensitivity, unstable color and rigid experimental conditions.

Assay of carisoprodol and its impurities with 2-methyl-2-propylpropane-1, 3-diyl dicarbamate and N-isopropyl-2-methyl-2-propyl-3-hydroxy propyl carbamate using UV-HPLC was presented by Rohith *et al.* [12], but has drawbacks in terms of precision, accuracy, retention time (16.855 min) and run time (50 min). In addition, the gradient mode of elution increases the use of solvents and the method is more concentrated on the characterization of impurities rather than the assay of carisoprodol. The main objective of the present investigation is to develop and validate a simple, sensitive, cost effective, selective and reproducible stability indicating HPLC method with UV detector for quantitative determination of carisoprodol and also to study the stability of carisoprodol. The present study is aimed to develop a reverse phase HPLC assay which is specific, precise, linear and accurate that can be used for routine analysis and stability study.

## MATERIALS AND METHODS

### Materials

HPLC grade acetonitrile was purchased from Merck India Limited, Mumbai, India. Analytical grade potassium dihydrogen phosphate, hydrochloric acid, sodium hydroxide and hydrogen peroxide were from Sdfine-Chem limited, Mumbai, India. Milli-Q-water was used throughout the process. The 2-Methyl-2-propyl-1,3-prpanediol; Carisoprodol Impurity A CRS Lot no: 1; Carisoprodol Sample and USP Carisoprodol RS were donated by Lee pharma as gift sample.

### Instrumentation

Separation and quantization of carisoprodol was performed on a High Pressure Liquid Chromatography (Waters, HPLC) equipped with 2345 Quaternary gradient pump and 2414 RI detector. The HPLC data were processed using Empower soft ware.

### Chromatographic conditions

The present study was aimed to carry out by reversed-phase chromatography to determine Carisoprodol; the column used was Sunfire-$C_{18}$ column (250 mm x 4.6 mm, 5 µm) at 30°C, mobile phase was prepared by mixing water with acetonitrile in the ratio 60:40v/v and refractive index detector at 30°C. The mobile phase was delivered at a flow rate of 1.0 ml per min. The mobile phase was filtered through a Millipore membrane filter paper and sonicated for 15 min for degassing prior to use.

**Preparation of standard solutions**

Water and acetonitrile in the ratio of 60:40 (*v/v*) is used as diluent for the preparation of standard solutions. A standard stock solution of carisoprodol (5 mg/ml) was prepared by dissolving 50.0 mg portion of USP Carisoprodol RS in 10-mL of mobile phase. Working standard solutions were prepared after the dilution of the stock solution with the same solvent. Five series of carisoprodol calibration solutions at the concentration values of 1, 2, 3, 4, 5 mg/ml were prepared from the stock standard solution by appropriate dilution with the diluent.

**Preparation of stress degradation samples**

Stress degradation samples were prepared using different ICH recommended stress conditions such as acidic, alkali, oxidative and thermal.

**Acid degradation**

For acid degradation, carisoprodol was dissolved in 5 ml of 1N Methanolic HCl in a 100 ml volumetric flask. The resulting solution was refluxed for 8 h at 60 °C on a heating mantle. After completion of the stress the solution was cooled and diluted to the volume with the diluents.

**Alkali degradation**

For alkali degradation, carisoprodol was dissolved in 5 ml of 0.1N Methanolic KOH for 1 h refluxing at 60 °C in a 100 ml volumetric flask. The resulting solution was refluxed for 1 h at 60 °C on a heating mantle. After completion of the stress the solution was cooled and diluted to the volume with the diluent.

**Oxidative degradation**

Oxidative degradation was carried out at 60 °C using 3% Ethanolic $H_2O_2$. To perform this, carisoprodol was dissolved in 5 ml of 3% Ethanolic $H_2O_2$ in a 100 ml volumetric flask. The resulting solution was refluxed for 24 h at 60 °C. After completion of the stress the solution was cooled and diluted to the volume with the diluent.

**Thermal degradation**

Thermal degradation studies were performed at 60 °C for 5 h. For this study, carisoprodol powder was taken in glass petric dish and placed under sunlight at 60 °C for 5 h. After specified time, the sample was cooled, transferred to a 100 ml volumetric flask and dissolved in 30 ml of diluent and made up to mark with the same solvent.

After degradation, all stress degraded samples were diluted to give a final concentration of 2 mg/ml and filtered through a millipore membrane filter paper before injection in the chromatographic system.

**Assay procedure**

The Assay preparation was dissolving 20.0 mg portion of sample in 10-mL of mobile phase.

**RESULTS AND DISCUSSION**

**Method development**

Initial trial, as suggested in the test plan was carried out by reversed-phase chromatography to determine Carisoprodol; the operating conditions were Sunfire-C18 column (250 mm x 4.6 mm, 5 µm) at 30 °C, mobile phase was prepared by mixing water with acetonitrile (60/40, v/v) and refractive index detector at 30 °C. The mobile phase was delivered at a flow rate of 1.0Ml per min. The diluent was prepared by mixing 0.01N Sulfuric acid and methanol in ratio of 40:60 (v/v). The sample concentration was 3.5 mg per ml of Carisoprodol in diluent. The peak response was good neither with the diluents nor with the mobile phase. Therefore, to increase the peak response, the column and detector temperature was increased to 45 °C (fig. 2).

Different concentrations were tried to get good peak shape and response. This was achieved with 5.0 mg/ml concentration of Carisoprodol and 20 µl injection load. Peak tailing of 1.48 and the LOD was found at a concentration of 0.015 mg per ml. Based on the equivalent concentration of 5.0 mg/ml concentration of Carisoprodol and 20 µl injection load, we checked with the concentration of 2.0 mg per ml and 50 µl injection load. The observed peak tailing of 1.32 and the LOD was found at a concentration of 0.01 mg per ml.

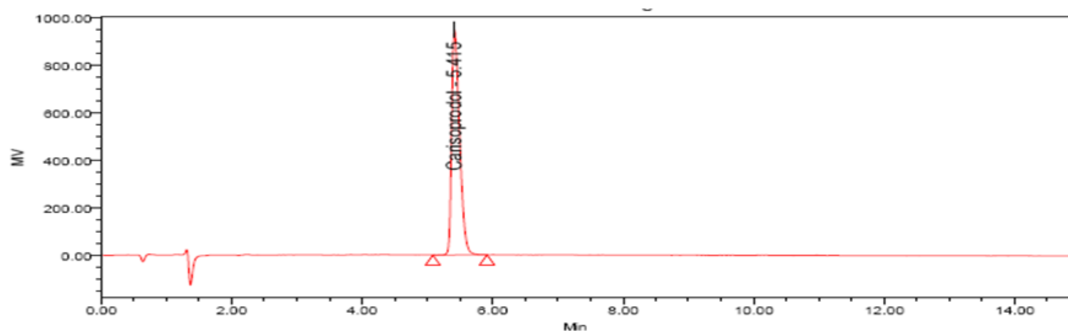


**Fig. 1: A representative chromatogram for carisoprodol concentration optimized to 5 mg/ml**

**Validation of the method**

The developed method was validated as per ICH guidelines by evaluating parameters like system suitability, linearity, limit of detection, limit of quantitation, precision, accuracy, specificity, robustness and ruggedness.

**System suitability**

**Carisoprodol impurity A stock solution**

An accurately weighed amount of 10.0 mg of carisoprodol Impurity A CRS Lot no: 1a into volumetric flask and dissolved it in mobile phase and diluted up to the mark with mobile phase.

**Carisoprodol impurity C stock solution**

An accurately weighed amount of 24.0 mg of 2-Methyl-2-propyl-1,3-prpanediol is dissolve in 10 ml of mobile phase.

An accurately weighed amount of 24.0 mg of Carisoprodol Impurity C (2-Methyl-2-propyl-1,3-prpanediol), 35.0 mg of USP Carisoprodol RS and 4 ml of Carisoprodol impurity A stock solution, are dissolved in 10 ml of Mobile phase.

Resolution between impurity C and impurity A of Carisoprodol to that of Carisoprodol is 18.0 and 2.34 respectively. The relative retention time for Carisoprodol impurity C was 0.4 and that of Carisoprodol Impurity A was 1.1 whereas for Carisoprodol it was 1.0. USP tailing for Carisoprodol peak for standard solution was 1.23 and % RSD for 5 replicate injections of standard solution was 0.42% (as shown in table 2).

**Assay precision**

The % relative standard deviation of carisoprodol from 6 sample preparations was 0.35% (table 2).

**Table 1: Performance parameters from the system suitability solution and standard solution**

| Parameter | Impurity C | Carisoprodol | Impurity A |
|---|---|---|---|
| $t_R$, min. | 2.2 | 5.3 | 5.8 |
| Relative $t_R$ | 0.4 | 1.0 | 1.1 |
| Resolution | 18.0 | | 2.3 |
| Tailing factor for Carisoprodol peak from standard solution | | 1.2 | |
| Repeatability from Standard solution | Mean peak area for replicate injections | 325768 | |
| | % RSD for replicate injections | 0.42 | |

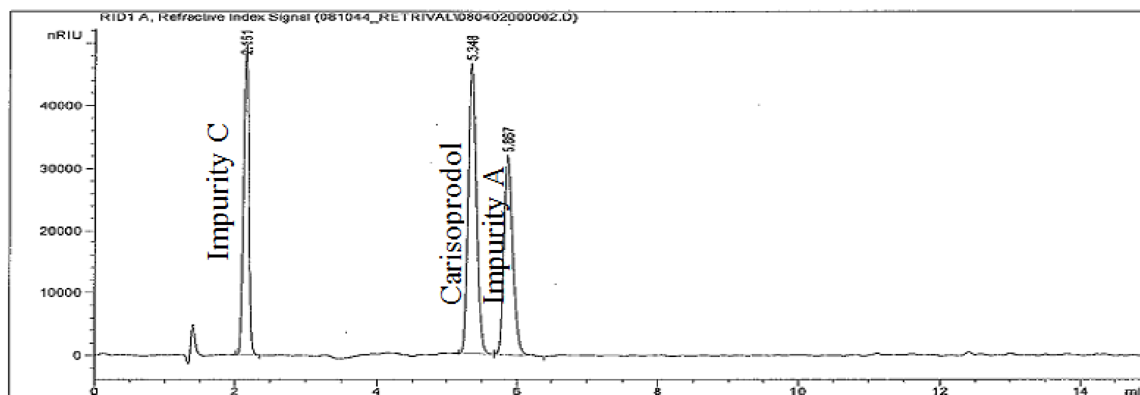


**Fig. 2: A representative chromatogram of a system suitability solution meets the requirements from precision**

**Table 2: The validation parameter, assay precision results**

| Preparations | % w/w of assay |
|---|---|
| Preparation-1 | 99.79 |
| Preparation-2 | 99.87 |
| Preparation-3 | 99.87 |
| Preparation-4 | 99.76 |
| Preparation-5 | 100.13 |
| Preparation-6 | 100.67 |
| mean±SEM (n=6) | 100.01±0.14 |
| % RSD | 0.35 |

**Linearity**

**Linearity stock solution:** A 200-mg portion of Carisoprodol is dissolve in 50 ml volumetric flask, dissolves and diluted to volume with mobile phase.

**Linearity solution 150%:** Pipette 15 ml of the linearity stock solution into 20 ml volumetric flasks, dilute to volume with mobile phase.

**Linearity solution 100%:** Pipette 25 ml of the linearity stock solution into 50 ml volumetric flasks, dilute to volume with mobile phase.

**Linearity solution 75%:** Pipette 15 ml of the Linearity solution 100%, into 20 ml volumetric flasks, dilute to volume with mobile phase.

**Linearity solution 50%:** Pipette 5 ml of the Linearity solution 100%, into 10 ml volumetric flasks, dilute to volume with mobile phase.

**Table 3: The validation parameter, linearity results**

| Concentration (in mg/ml) | Mean area |
|---|---|
| 1.002 | 162831 |
| 1.503 | 246392 |
| 2.004 | 331649 |
| 3.006 | 496392 |
| 4.007 | 662755 |
| Correlation coefficient ($R^2$) | 0.99998 |

**Accuracy**

Assay accuracy preparations at 80%, 100% and 120% level for Carisoprodol: Weigh 40.0 mg, 50.0 mg and 60.0 mg of Carisoprodol, each in triplicate, into a 25 ml of volumetric flask, dissolve it and diluted to volume with mobile phase.

The accuracy of the method is determined by recovery experiments. The recovery was performed by Carisoprodol sample at 80%, 100% and 120% levels against USP Carisoprodol RS. At each level, three sample preparations were used. The average % recovery of these three preparations at their respective concentration level was 100.55%, 100.67% and 101.11% respectively. The results were found to be within the desired acceptance criteria according to which, the % Recovery should not be less than 98.0% and not be more than 102.0 %. Typical chromatograms of both Standard and assay preparations are shown in fig. 6 to fig. 8. The assay data are listed in table 4.

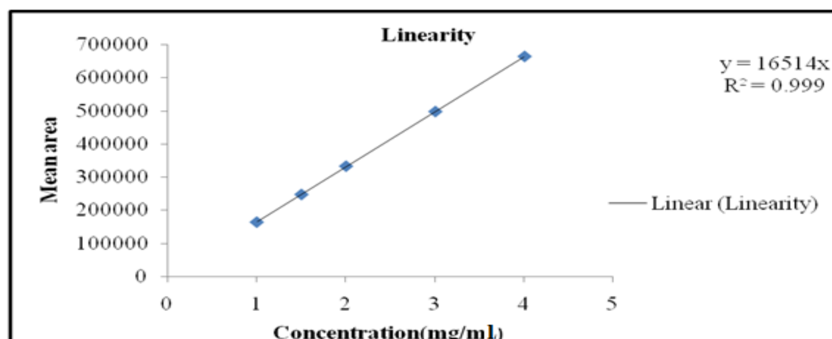


**Fig. 3: Calibration curve for carisoprodo l from linearity study**

**Table 4: The validation parameter, accuracy results**

| Level of accuracy | S. No. | Drug added (in mg) | Drug recovered (in mg) | % assay mean±SEM (n=3) | % RSD of assay, (n=3) |
|---|---|---|---|---|---|
| at 80% | 1 | 40.49 | 40.79 | 100.55±0.27 | 0.97 |
| | 2 | 40.20 | 40.23 | | |
| | 3 | 40.18 | 40.03 | | |
| at 100% | 1 | 50.30 | 50.19 | 100.67±0.39 | 0.67 |
| | 2 | 50.05 | 50.09 | | |
| | 3 | 50.52 | 50.72 | | |
| at 120% | 1 | 60.63 | 60.39 | 101.11±0.23 | 0.39 |
| | 2 | 60.50 | 60.79 | | |
| | 3 | 60.44 | 60.81 | | |

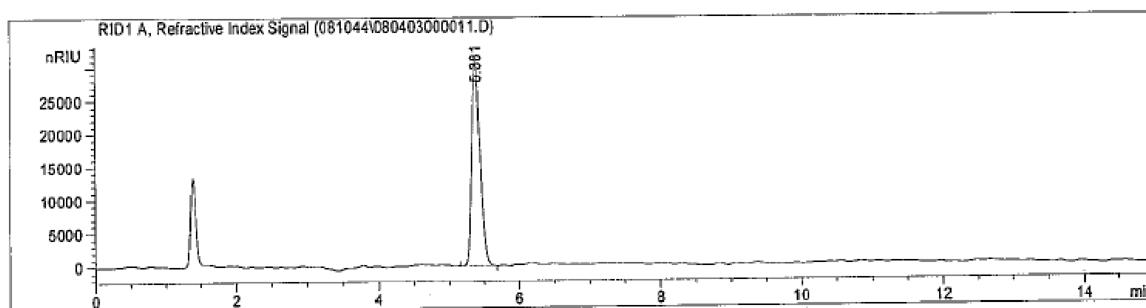


**Fig. 4: A representative chromatogram of an accuracy 80% solution exhibit carisoprodol peak**

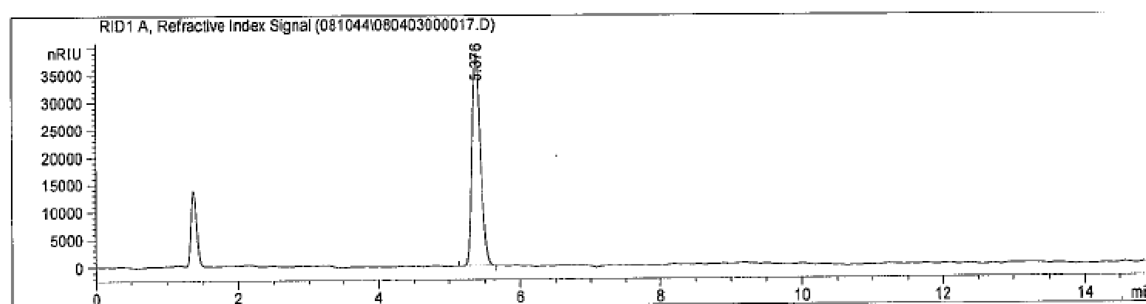


**Fig. 5: A representative chromatogram of an accuracy 100% solution exhibit carisoprodol peak**

**Stability of mobile phase**

The % RSD of retention times for different intervals of 0h, 24h, 30h and 48h was found to be 0.13% and similarly, the assay results for fresh sample preparations in different intervals was found to be 0.58% (table 5).

**Sample solution stability**

The % RSD for Assay results of initial sample preparation in different intervals of 0hr, 24 h, 30 h and 48 h was found to be 0.46% (table 5).

**Degradation studies**

From the forced degradation studies, it was observed that carisoprodol was quickly degraded to impurity A in alcoholic alkaline media which was confirmed by injecting the carisoprodol impurity A CRS Lot no: 1a solution into chromatographic system, and resolution between carisoprodol peak and Impurity A obtained was 2.3. The RRT of impurity A was 1.1.

From table no: 8 of degradation studies, in 0.1N methanolic KOH, the controlled degradation of carisoprodol in 0.1N methanolic KOH for 1 h refluxing at 60 °C, showed 10.6% degradation as

shown in fig. 7. In 1N Methanolic HCl, refluxing for 8 h at 60 °C, the degradation of Carisoprodol in 0.1N methanolic HCl showed 5.1% degradation as shoen in fig. 8 and in 3% ethanolic $H_2O_2$, the degradation of carisoprodol in 3% ethanolic $H_2O_2$ for 24 h reflux at 60 °C, showed 9.1% degradation which is shown in fig. 9. Upon Thermal exposure, no degradation was observed from solution form as well as powder form of carisoprodol at 60 °C for 24 h (fig. 10-13).

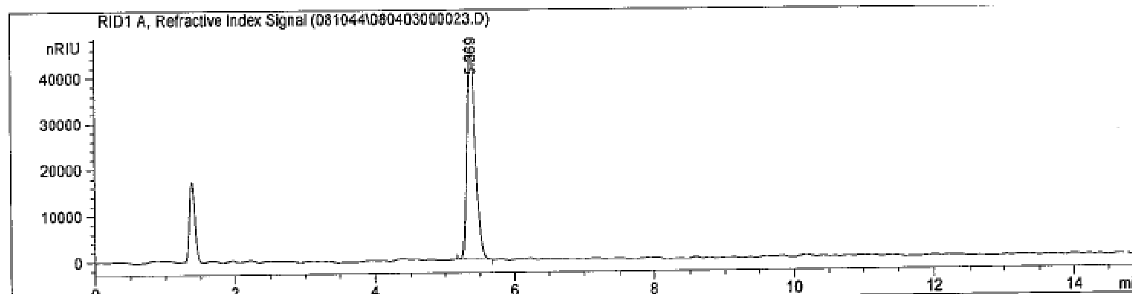


**Fig. 6: A representative chromatogram of an accuracy 120% solution exhibit carisoprodol peak**

**Table 7: The validation parameter, stability of mobile phase and analyte solution results**

| Time interval | Retention time (in min) | Mobile phase stability | Analyte solution stability |
|---|---|---|---|
| 0 H (Initial) | 5.23 | 99.69 | 99.69 |
| 24 H | 5.25 | 99.92 | 99.51 |
| 30 H | 5.25 | 99.17 | 100.45 |
| 48 H | 5.24 | 100.58 | 99.47 |
| mean±SEM (n= 4) | 5.24±0.00 | 99.84±0.34 | 99.78±0.22 |
| %RSD | 0.13 | 0.58 | 0.46 |

**Table 8: Representing controlled degradation for Forced degradation study**

| Stress condition | % degradation | Optimized time |
|---|---|---|
| 1 N Methanolic Hcl, at 60 °C | 5.1 | 8 h |
| 0.1N Methanolic KOH, at 60 °C | 10.6 | 1hr |
| 3% $H_2O_2$, at 60 °C | 9.1 | 24 h |
| Heat at 60 °C | 0 | 24 h |
| UV-short wave length | 0 | 24 h |
| Sunlight | 0 | 5 h |

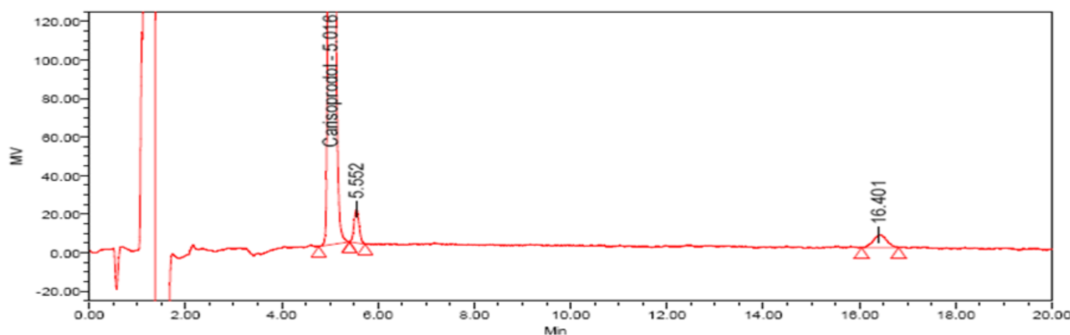


**Fig. 7: A representative chromatogram of about 5% degradation in stress study for carisoprodol in acid media (1N Hcl, Methanolic-8h reflux, at 60 °C)**

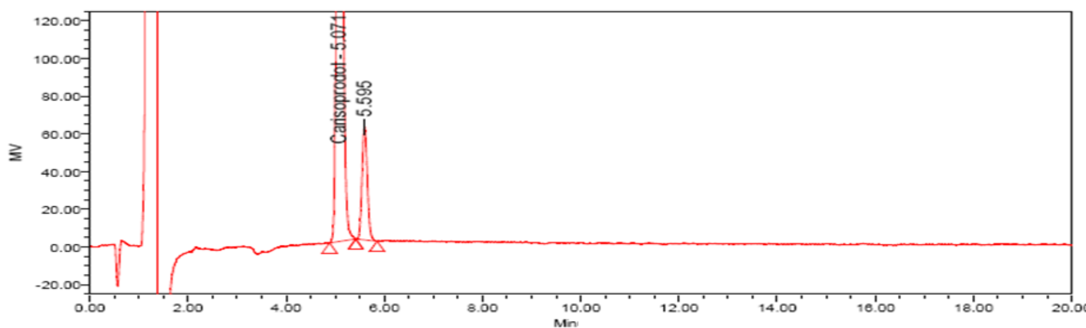


**Fig. 8: A representative chromatogram of about 10% degradation in stress study for carisoprodol in acid media (1N KOH, methanolic-1h reflux, at 60 °C)**

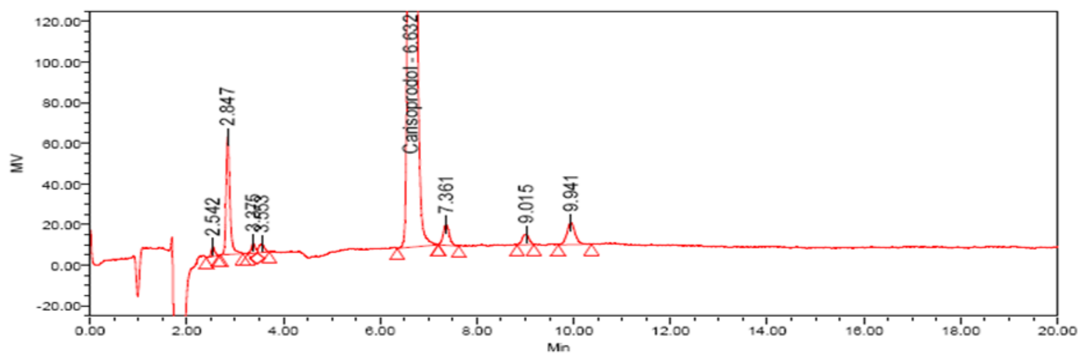


**Fig. 9: A representative chromatogram of about 10% degradation in stress study for carisoprodol in acid media (3%$H_2O_2$, alcoholic-24h reflux, at 60 °C)**

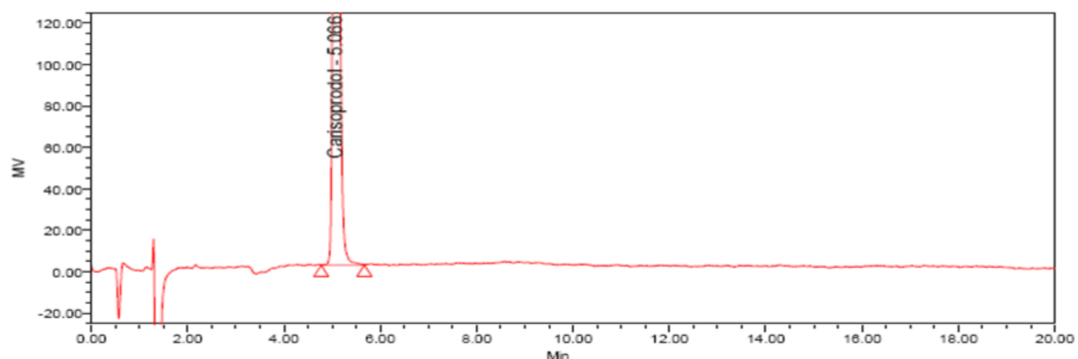


**Fig. 10: A chromatogram representing no degradation in carisoprodol test solution heated at 60 °C for 24 h during stress study**

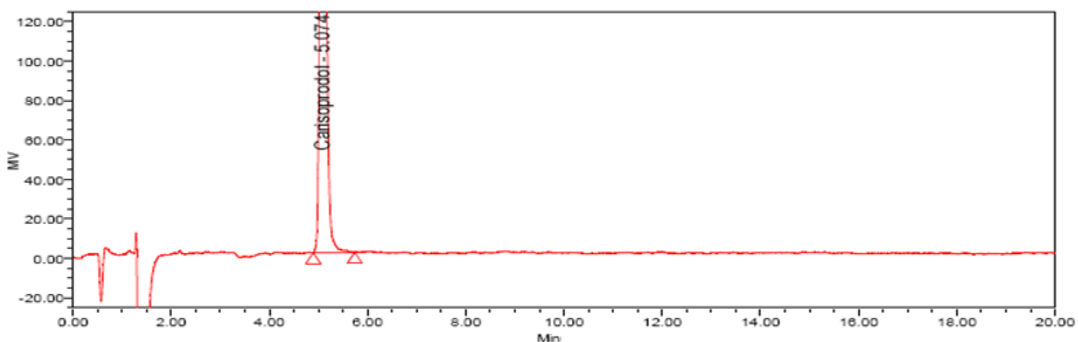


**Fig. 11: A chromatogram representing no degradation in carisoprodol powder, heated at 60 °C for 24 h during stress study**

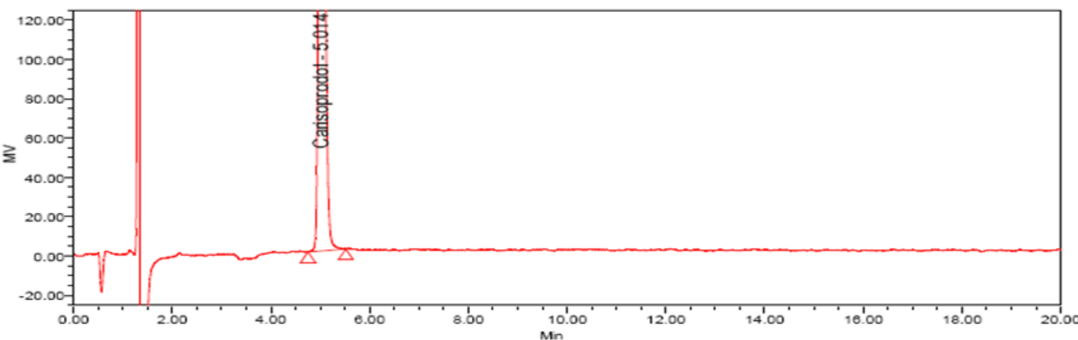


**Fig. 12: A chromatogram representing no degradation in carisoprodol test solution under sunlight for 5 h during stress study**

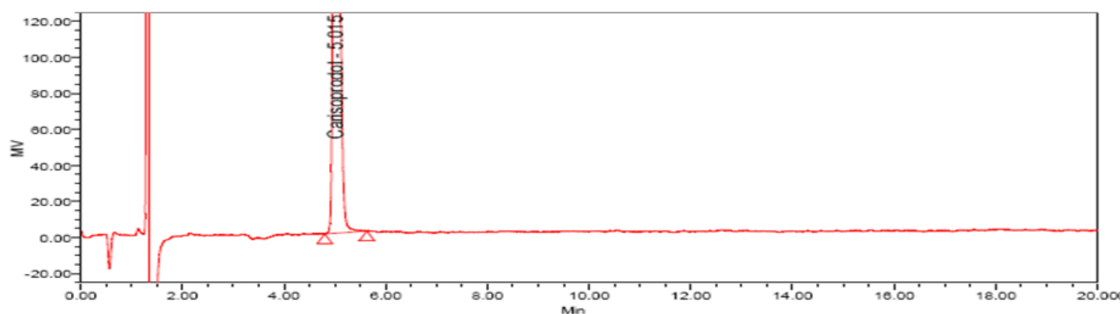


**Fig. 13: A chromatogram representing no degradation in carisoprodol powder under sunlight for 5 h during stress study**

## CONCLUSION

The Assay method adopted for Carisoprodol is specific, precise, linear and accurate. The Analyte solution was found to be stable up to 48 h under ambient conditions and mobile phase was found to be stable up to 48 h. Hence this method can be used to replace the existing titration method for routine analysis and stability study of carisoprodol.

## CONFLICT OF INTERESTS

Declared none

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