International Standard Serial Number (ISSN): 2319-8141
*International Journal of Universal Pharmacy and Bio Sciences 2*(3)*: May-June 2013*
***INTERNATIONAL JOURNAL OF UNIVERSAL PHARMACY AND BIO SCIENCES***

**Pharmaceutical Sciences** **Research Article……!!!**

Received: 23-04-2013; Accepted: 01-05-2013

# DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR SIMULTANEOUS ESTIMATION OF CARISOPRODOL AND ASPIRIN IN BULK AND SYNTHETIC MIXTURE BY ABSORPTION RATIO METHOD USING 1, 2 NAPTHAQUINONE 4 SULPHONIC ACID SODIUM SALT

Vandita Patel, Hemant Patel, Meha Patel

Babaria Institute of Pharmacy BITS Edu Campus

Vadodara-mumbai NH#8, Varnama, Vadodara, Gujarat, India

**KEYWORDS:**

Carisoprodol (CAR), Aspirin (ASP), Derivatization, Validation.

**For Correspondence:**

**Vandita Patel ***

**Address:** Babaria Institute of Pharmacy BITS Edu Campus

Vadodara-mumbai NH#8, Varnama, Vadodara, Gujarat, India.

Mob. No. 07874359770.

**ABSTRACT**

A simple and sensitive spectrophotometric method has been developed for absorption ratio of Aspirin and Carisoprodol in a combined pharmaceutical dosage form. The proposed method is based on the derivatization of Aspirin and Carisoprodol by reagent 1, 2 naphthoquinone 4 sulphonic acid sodium salt in the presence of borate buffer. Linearity range was observed in the concentration range of 50-90 µg/ml for Carisoprodol and 50-90 µg/ml for Aspirin. The method involved Q-absorption analysis based on the measurement of absorbance at two wavelengths, i.e λmax of Aspirin (295.0 nm) and Iso-absorptive point of both drugs (326.0 nm). The method was validated statistically and recovery study was performed to confirm the accuracy of the method. The method was found to be rapid, simple, accurate and precise.

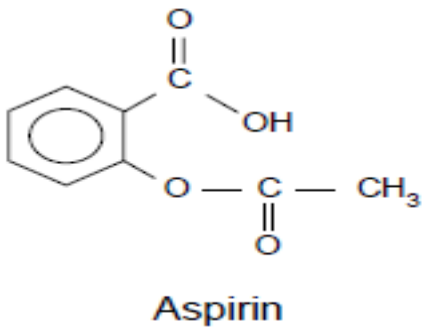
**INTRODUCTION [1-3]**:

Aspirin is salicylate drug which have non-narcotic analgesic, anti-inflammatory and antipyretic activity. It is used to relieve minor patches and pain. The mechanism of action of aspirin in relieving pain is by inhibition of the body's production of prostaglandins, which are thought to cause pain sensations by stimulating muscle contractions and dilating blood vessels. In the CNS, aspirin works on the hypothalamus heat-regulating center to reduce fever.

Aspirin is widely used drug alone and with its combination. Aspirin is official in Indian pharmacopoeia, British Pharmacopoeia, United state pharmacopoeia. Many analytical methods are reported for aspirin like High performance liquid chromatography, titration, GC/MS and LC/MS, Spectroscopy.

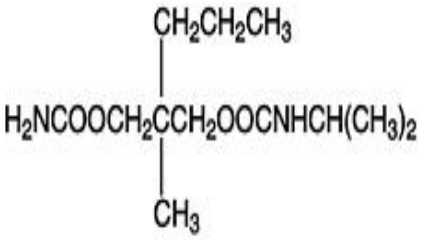
Carisoprodol is a dicarbamate, centrally acting, oral skeletal muscle relaxant whose chief application is in the treatment of acute muscular spasm associated with craniomandibular disorder, lumbago, sciatica, and other lower back syndromes.

Carisoprodol is a centrally acting skeletal muscle relaxant that does not directly relax tense skeletal muscles in man. The mode of action of carisoprodol in relieving acute muscle spasm of local origin has not been clearly identified, but may be related to its sedative properties. Carisoprodol is official in British Pharmacopoeia and United state pharmacopoeia but few analytical methods are available for Carisoprodol.




Aspirin

2 Acetoxybenzoic acid




N-isopropyl-2-methyl-2-propyl-1,3-propanediol dicarbamate

**Figure 1:Structure of aspirin**

**Figure 2: Structure of Carisoprodol**

**MATERIALS AND METHODS [4-6]:**

**INSTRUMENT:**

A Shimadzu model UV-1800 double beam UV-visible Spectrophotometer, attached to a computer software UV probe 2.34, with a spectral width of 1 nm and pair of 1 cm matched

quartz cells was used. Shimadzu analytical balance (Sartorius, Gottingen, Germany), and Ultrasonic cleaner (Frontline FS 4, Mumbai, India).pH meter CL54+ (Toshcon industries pvt.ltd) were used throughout the practical. Class 'A' volumetric glassware were used.

**REAGENTS AND MATERIALS:**

Carisoprodol and Aspirin bulk powder was kindly gifted by Centurian laboratories in, Vadodara(India), with 99.96% purity. The commercial fixed dose combination product ( containing 200 mg CAR and 325 mg ASP).

The alkaline borate buffer solutions (0.1M) were made by dissolving boric acid in water and adjusted to the desired pH with 2 M NaOH solution.

Stock solution of 1,2 naphthoquinone 4 sulphonic acid sodium salt was freshly prepared by dissolving 0.2 g in 100 mL distilled water and stored in the dark (a flask coated with aluminum foil) at room temperature.

**PREPARATION OF STANDARD STOCK SOLUTIONS:**

Accurately weighed portions of CAR (50 mg) and ASP (50 mg) were transferred to a separate 100ml volumetric flask and dissolved and diluted to the mark with Methanol to obtain standard solution having concentrations of CAR(500 µg/ml) and ASP (500 µg/ml). The standard solutions were prepared by further dilution of the stock standard solution with the specified borate buffer to reach the concentration range of 50- 90 µg/ml for Carisoprodol and 50- 90 µg/ml Aspirin.

**PREPARATION OF SAMPLE SOLUTION:**

Take 80 mg of aspirin and 50 mg of Carisoprodol and transferred in to a 100 ml volumetric flask. The solution was filtered through whatman filter paper No. 41 and the volume was adjusted up to the mark with methanol. This solution is expected to contain 800 µg/ml aspirin and 500 µg/ml carisoprodol. Solutions were prepared by further dilution of this solution with the specified borate buffer to reach the concentration of 50 µg/ml for Carisoprodol and 80 µg/ml Aspirin.

**PREPARATION OF CALIBRATION CURVE:**

Standard solutions of Aspirin (1.0, 1.2, 1.4, 1.6, 1.8 ml) and standard solutions of Carisoprodol (1.0, 1.2 1.4, 1.6, 1.8 ml) was pipette out in to a separate series of 10 ml volumetric flask. To each flask 0.5 ml(100 µg/ml) of NQS and 1 ml of Borate buffer (pH 8.5 ,0.1 M) were added. The solutions were allowed to stay for 20 min in a thermostated oven at $70^0$ C, cooled to room temperature. The content of each flask was diluted up to the mark with Borate buffer. The absorbances of the solutions were measured at 326.0 nm and 295.0 nm against reagent blank.

The Lambert-Beer's law was obeyed in concentration range of 50 to 90 µg at 326.0 nm and 50 to 90 µg at .0 295 nm for Aspirin and 50 to 90 µg at 326.0 nm and 50 to 90 µg at 362.0 nm for Carisoprodol.

**DEVELOPMENT OF THE METHOD [4-8]:**

**Q-Absorption Ratio Method:** This method is applicable to the drugs that obey Beer's law at all wavelengths and the ratio of absorbances at any two wavelengths are a constant value, independent of concentration or path length. Two wavelengths, 326 nm (Isoabsorptive point) and 295 nm (λmax of ASP) were selected for the formation of Q-absorbance equation. The absorptivity co-efficient of each drug at both the wavelengths were determined. The concentration of individual components, ASP and CAR may be calculated using the following equations

CASP= (Qm–QCAR/QASP–QCAR)*A1/ax1 …….. (1)

CCAR= (Qm–QASP/QASP–QCAR)*A1/ay1 …….. (2)

Where, Qm= A2 / A1, QASP = ax2 / ax1 & QCAR = ay2/ay1; A1 and A2 are absorbance of sample solution at Isoabsorptive point (326.0 nm) and λmax of ASP (295 nm) respectively; ax1 and ax2 are the absorptivities of ASP at 326.0 and 295.0 nm respectively and ay1 and ay2 are the absorptivities of CAR at the two wavelengths respectively.

**METHOD VALIDATION [5]:**

**LINEARITY:**

Calibration curves were plotted over a concentration range of 50-90 µg/ml and 50-90 µg/ml for ASP and CAR respectively. Accurately measured standard working solutions of ASP (1.0, 1.2, 1.4, 1.6, 1.8 ml) and CAR (1.0, 1.2, 1.4, 1.6, 1.8 ml) were transferred to a series of 10ml of volumetric flasks and To each flask 0.5 ml(100 µg/ml) of NQS and 1 ml of Borate buffer (pH 8.5 ,0.1 M) were added. The solutions were allowed to stay for 20 min in a thermostated oven at $70^0$ C, cooled to room temperature. The volumes were made up to the mark with borate buffer and absorbance were measured at 326.0 nm and 295.0 nm for both drugs. The calibration curves were constructed by plotting absorbances Vs concentrations(Figure-2, 3, 4&5 and Table-1).

**METHOD PRECISION (REPEATABILITY):**

The precision of the instrument was checked by repeated scanning and measurement of the absorbance of solutions (n = 6) of ASP and CAR (70µg/ml and 70µg/ml) without changing the parameters for the ABSORTION RATIO method.

**INTERMEDIATE PRECISION (REPRODUCIBILITY):**

The intraday and interday precisions of the proposed methods were determined by analyzing the corresponding responses 3 times on the same day and on 3 different days with 3 different concentrations of standard solutions of ASP and CAR (50, 70, and 90 µg/ml and 50, 70, 90 µg/ml respectively). The results were reported in terms of relative standard deviation (RSD). (Table-4)

**LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION:**

The limit of detection (LOD) and limit of quantification (LOQ) of the drug wee derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ ) ratio using the following equations designated by International Conference on Harmonization (ICH) guideline(Table-2):

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, $\sigma$ = the standard deviation of the response

S = slope of the calibration curve.

**ACCURACY (RECOVERY STUDY):**

The accuracy of the methods was determined by calculating recoveries of ASP and CAR by the standard addition method. Known amounts of standard solutions of ASP and CAR were added at 80%, 100% and 120% levels to prequantified sample solutions of ASP and CAR(70 and 70 µg/ml respectively). The amounts of ASP and CAR were estimated by applying the obtained values to the Q ratio method (Table-3).

**ANALYSIS OF ASPIRIN AND CARISOPRODOL IN SYNTHETIC MIXTURE:**

Binary mixture was prepared for combination of both drug in ratio of 80 : 50(ASP and CAR). The absorbance was measured at 326.0 and 429.0 nm for quantification of ASP and CAR, respectively. The amounts of ASP and CAR present in sample solutions were determined by fitting the response into the simultaneous equation for ASP and CAR. No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of Aspirin and Carisoprodol in mixture.

**RESULTS AND DISCUSSION:**

The structure of Carisoprodol is such that it does not have a UV chromophore with significant absorbance. Thefore derivatization for the purpose of adding strong UV chromophore to the compound is must for its analysis. From that point 1,2 naphthoquinone 4 sulphonic acid sodium salt which is the colour labelling reagent for primary amines was used as derivatizing agent for Carisoprodol.

After the derivatization maximum absorbance was obtained at 362 nm and 295 nm for Carisoprodol and Aspirin respectively. Select this two wavelengths for determination of Carisoprodol and Aspirin.

The developed methods were optimized using different parameters such as reaction time, reaction temperature and NQS concentration. The optimization was executed with the 0.5 ml of NQS at 100 mg/mL, 0.1 M borate buffer, pH at 8.5 for 20 min at $70^0$c.

In this method, the overlain spectra of drugs showed the isoabsorptive point at 326 nm and λmax of ASP at 295 nm. Both the drugs obeyed linearity range 50-90 µg/ml and 50-90 µg/ml respectively and correlation coefficient ($r^2$) were found to be <1 in both cases. The absorptivity values were calculated and along with absorbances, these values were submitted in equation and to obtain concentration of drugs. The accuracy of the method was determined by performing recovery study by standard addition method. The experiment was repeated six times in a day for precision. The method was found to be precise as % RSD for precision were <2.

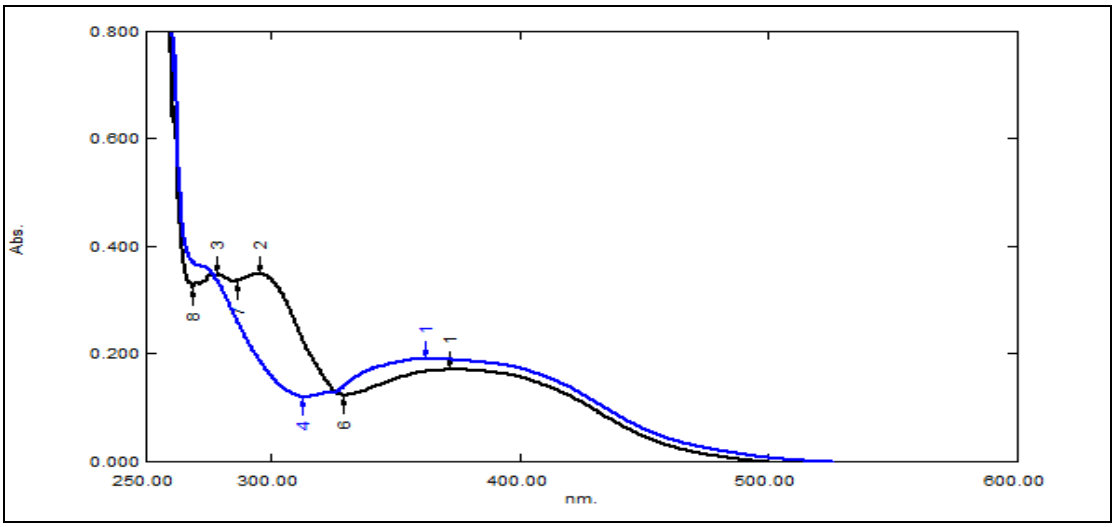



**Figure 3: overlain spectra of aspirin and carisoprodol:**

## VALIDATION OF THE PROPOSED METHOD:

### Linearity

Linear correlation was obtained between absorbance versus concentrations of ASP and CAR in the ranges of 50-90µg/ml and 50-90 µg/ml, respectively. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression (Table.1, Fig. 4, 5, 6 and 7 ).

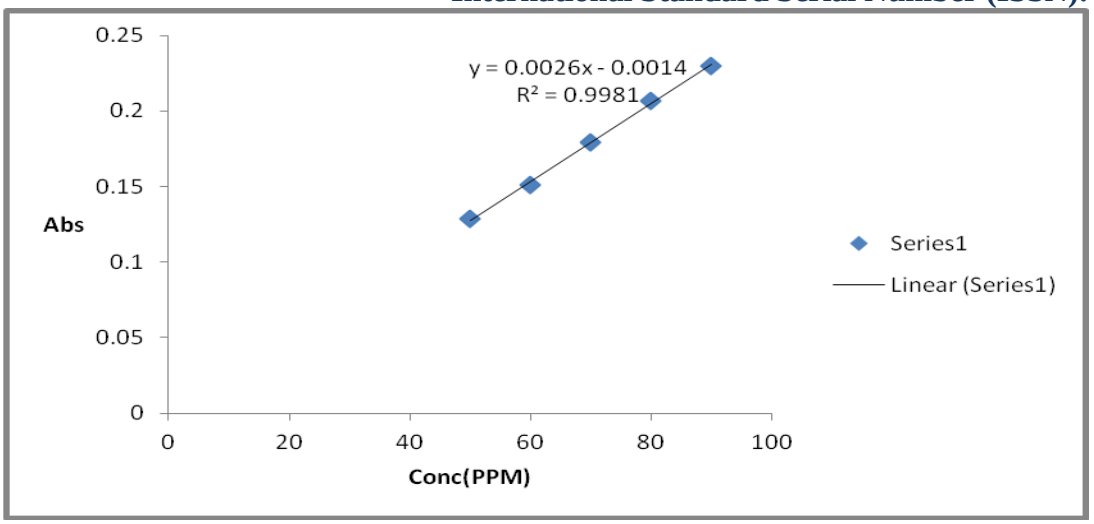



**Figure 4:Calibration Curve of Aspirin at 327 nm**

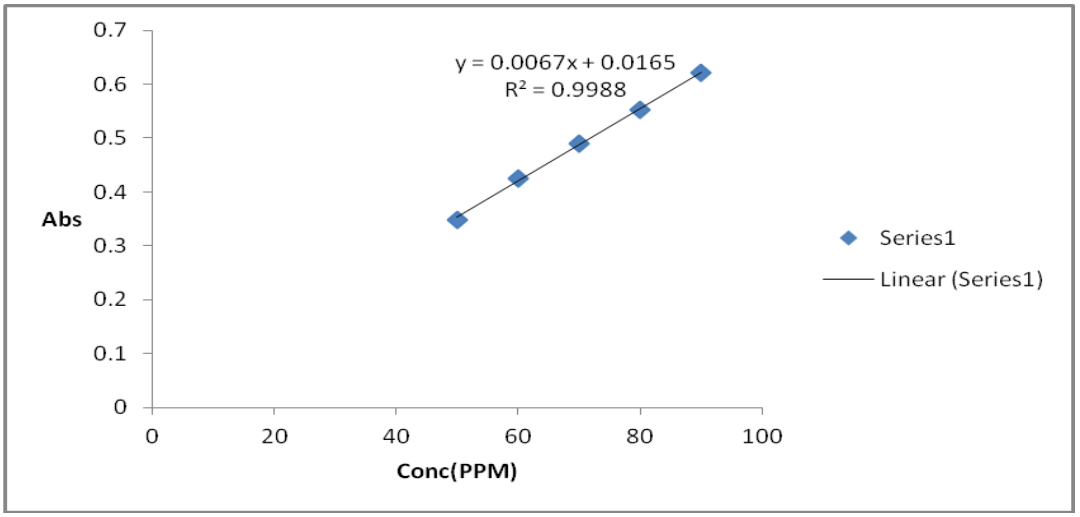



**Figure 4: Calibration Curve of Aspirin at 295 nm**

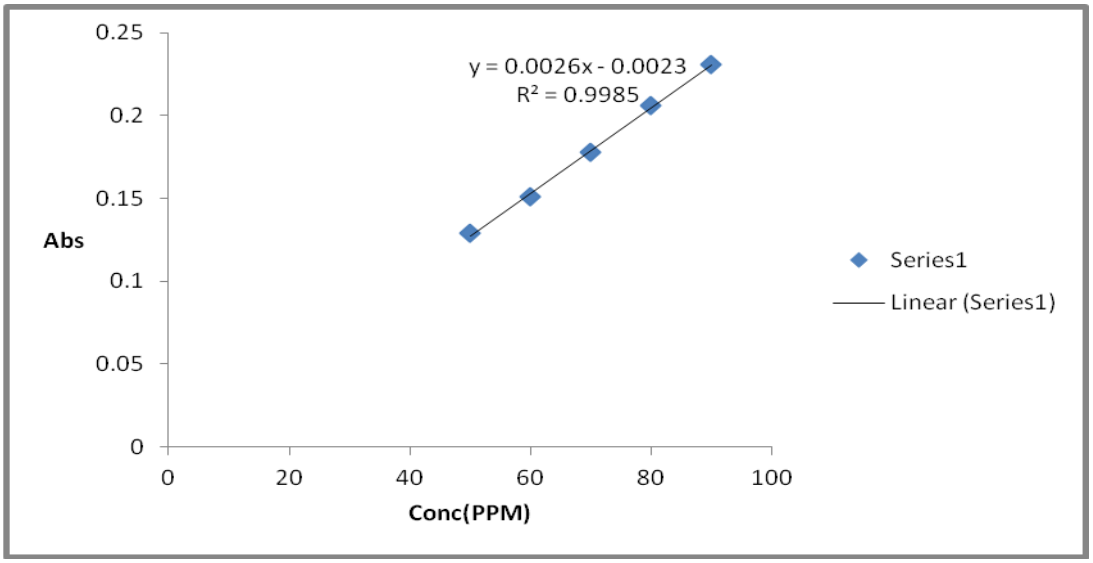



**Figure 5: Calibration Curve of Carisoprodol at 327 nm**

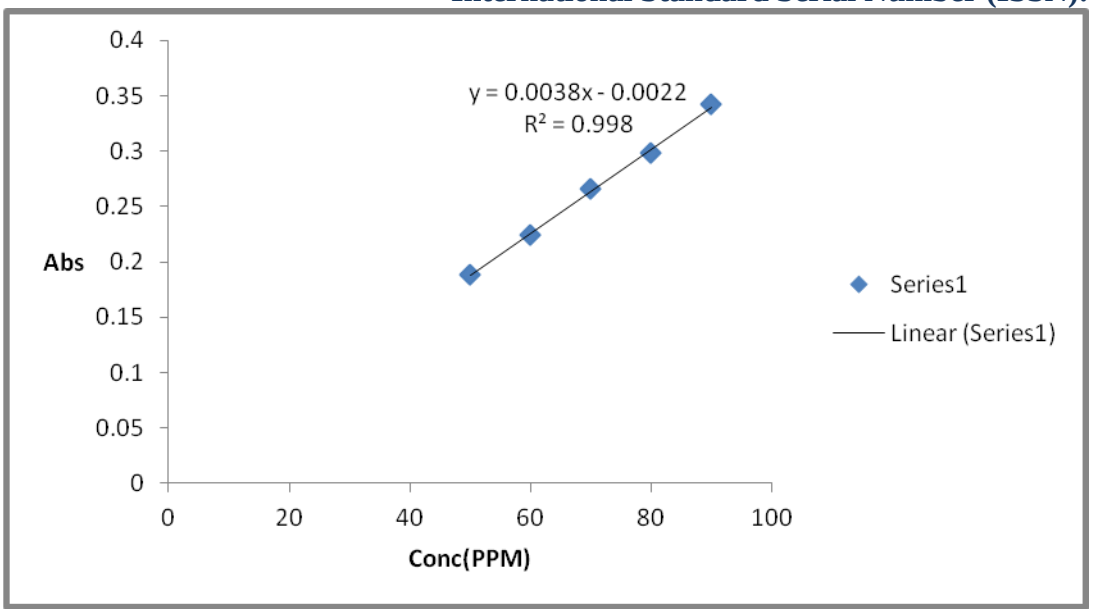



**Figure 6: Calibration Curve of Carisoprodol at 295 nm**

**TABLE 1: REGRESSION ANALYSIS DATA AND SUMMARY OF VALIDATIONPARAMETER FOR THE PROPOSED METHOD:**

| **Parameters** | **ASPIRIN** | | **CARISOPRODOL** | |
|---|---|---|---|---|
| Wavelength (nm) | 326.0 | 295.0 | 326.0 | 295.0 |
| Beer's Law Limit (µg/ml) | 50-90 | 50-90 | 50 – 90 | 50 – 90 |
| Regression equation (y= a + bc )<br>Slope (b)<br>Intercept (a) | <br>0.006<br>0.016 | <br>0.002<br>0.001 | <br>0.003<br>0.002 | <br>0.002<br>0.002 |
| Correlation Coefficient ($r^2$) | 0.998 | 0.998 | 0.998 | 0.998 |

**LOD and LOQ**

LOD and LOQ values for Aspirin were found to be 0.451 and 1.36 µg/ml. Where, LOD and LOQ values for Carisoprodol were found to be 0.485 and 1.475 µg/ml, and (Table- 2). These data show that method is sensitive for the determination of Aspirin and Carisoprodol.

**Method precision (repeatability)**

The RSD values for Aspirin and Carisoprodol were found to be 0.584% and 0.333 % respectively. Relative standard deviation was less than 2 %, which indicates that the proposed method is repeatable.

**TABLE 2: LOD AND LOQ PARAMETER FOR THE PROPOSED METHOD**

| **Parameters** | **ASPIRIN** | **CARISOPRODOL** |
|---|---|---|
| LOD(µg/ml) | 0.451 | 0.485 |
| LOQ(µg/ml) | 1.3693 | 1.471 |
| Repeatability (n=6)% | 0.584 | 0.333 |

**INTERMEDIATE PRECISION (REPRODUCIBILITY):**

The low RSD values of interday (0.37 – 0.89 % for Aspirin and 0.77 – 1.09% for Carisoprodol and intraday (0.20-0.54 % for Aspirin and 0.20-1.00% for Carisoprodol, respectively) variations for ASP and CAR, reveal that the proposed method was precise (Table 3).

**TABLE 3: INTER-DAY AND INTRA-DAY PRECISION:**

| **Inter day** | | | | | | **Intra day** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Amount taken(PPM)** | | **Amount Found(PPM)** | | **%RSD** | | **Amount taken(PPM)** | | **Amount Found(PPM)** | | **%RSD** | |
| **ASP** | **CAR** | **ASP** | **CAR** | **ASP** | **CAR** | **ASP** | **CAR** | **ASP** | **CAR** | **ASP** | **CAR** |
| 50 | 50 | 50.08 | 49.89 | 0.37 | 0.77 | 50 | 50 | 49.81 | 49.49 | 0.54 | 01.00 |
| 70 | 70 | 69.98 | 68.75 | 0.78 | 1.09 | 70 | 70 | 69.73 | 69.14 | 0.35 | 0.628 |
| 90 | 90 | 90.06 | 89.55 | 0.89 | 1.07 | 90 | 90 | 89.52 | 89.83 | 0.20 | 0.202 |

**ACCURACY**

The recovery experiments were performed by the standard addition method. The mean recoveries were 100.22 ± 0.614 and 99.91 ± 1.18 % for Aspirin and Carisoprodol, respectively (Table 4.1). The low value of standard deviation indicates that the proposed method is accurate. Results of recovery studies are shown in Table 4.

**TABLE 4: RECOVERY DATA FOR THE PROPOSED METHOD (n=5)**

| **Drug** | **Amount present in mixture(µg/ml)** | **Amount Added (%)** | **% Recovery ±SD** |
|---|---|---|---|
| **ASP** | 70 | 80 % | 100.22%±0.614 |
| | 70 | 100 % | 99.05%±0.677 |
| | 70 | 120 % | 99.54%±0.427 |
| **CAR** | 70 | 80 % | 99.21%±1.18 |
| | 70 | 100 % | 99.38%±0.616 |
| | 70 | 120 % | 98.56%±0.318 |

**TABLE 5: ANALYSES OF ASPIRIN AND CARISOPRODOL BY PROPOSED METHOD**

| Sample No. | Amount Taken | | Amount Found | | % Found | |
|---|---|---|---|---|---|---|
| | ASP (PPM) | CAR (PPM) | ASP (PPM) | CAR (PPM) | ASP | CAR |
| **1** | 80 | 50 | 79.83 | 49.78 | 99.78 | 99.56 |
| **2** | 80 | 50 | 79.68 | 50.32 | 99.6 | 100.64 |
| **3** | 80 | 50 | 80.15 | 49.46 | 100.18 | 98.92 |
| **4** | 80 | 50 | 80.32 | 49.68 | 100.4 | 102.82 |
| **5** | 80 | 50 | 79.37 | 51.41 | 99.21 | 103.26 |
| **6** | 80 | 50 | 79.54 | 51.63 | 99.76 | 100.76 |
| **Mean** | | | 79.815 | 50.38 | 99.76 | 100.76 |

**ASSAY OF THE SYNTHETIC MIXTURE:**

The proposed validated methods were successfully applied to determine Aspirin and Carisoprodol in their combined dosage forms. Results are given in table 4.4. No interference of the excipients with the absorbance of interest appeared; hence the proposed method applicable for the routine simultaneous estimation of Aspirin and Carisoprodol in mixture.

**CONCLUSION**

Based on the results, obtained from the analysis of using described method, it can be concluded that the method has linear response in the range of 50– 90 and 50 – 90 µg/ml for Aspirin and Carisoprodol.

The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and is in good agreement with label claim of the drugs. The additive usually present in the pharmaceutical formulations of the assayed samples did not interfere with determination of Aspirin and Carisoprodol. The method can be used for the routine analysis of Aspirin and Carisoprodol in combined dosage form.

**ACKNOWLEDGEMENT:**

The authors are thankful to Centurion Laboratories Pvt. Ltd. and Babaria Institute of Pharmacy, Vadodara, Gujarat, India for providing Aspirin and Carisoprodol for research. The authors are highly thankful to Babaria Institute of Pharmacy, Vadodara, and Gujarat, India for providing all the facilities to carry out the work.

**REFERENCES**

1. Soma, Carisoprodol, Rx list monograph Available from: URL : http://www.rxlist.com/soma-drug.htm Accessed on 2012 July 31.

2. Carisopridol, Drug update available from: URL : http://www.drugsupdate.com/generic/view/761
3. Bayer ASA, Aspirin, Rx list monograph Available from: URL: Http://www. rxlist.com/cgi/generic/asa.htm Accessed on 2006 Feb17.
4. Beckett, A. H., Stenlake, J. B., Practical Pharmaceutical Chemistry, Part-II, 4th Edn., CBS Publishers and distributors, India, 1997.
5. ICH, Q2 (R1) Validation of Analytical Procedure, Text and Methodology, International Conference on Harmonization; 2005 .
6. Sharma Sangita, Madhurjya neog, (2010), Spectrophotometric estimation of sulfadoxine in pharmaceutical preparations; Journal of chemistry, 7(4); 1246-1253.
7. US Pharmacopeia 34 NF 29. The United State Pharmacopoeial Convention, Rockvill, 2011
8. British Pharmacopoeia-2009, pp. 3
9. Indian Pharmacopoeia. New Delhi: Ministry of health and Family welfare; 2007.vol II,127-129
10. Willard Merritt, Dean Settle, Instrumental method of chemical analysis,7th Ed.593–600.
11. Jin Young K, Moon In K, Ki J.P, Bong C.C; (2005), Simultenous determination of Carisoprodol and Meprobromate in human hair using solid phase extraction and gas chromatography/mass spectroscopy of the trimethysilyl derivatives, Rapid communationsin Mass Spectroscopy; 3056-3062.
12. Irina R.M and Miksa R.H; (2008), Direct and rapid determination of Baclofen and Carisoprodol in Bovine serum by liquid chromatography mass spectroscopy; Journal of analytical toxicology; Aug 2008, vol-27; 275-283.
13. Suresh Kumar S., Latif D.J, Bhat K, Musmade P.B, Vasantharaju S.G, Udupav. N; (2010), Analytical method development and validation of Aspirin; International journal of chemtech research; 389-399.
14. Hadad G.M, Abdel-Salam R.A, Emara S; (2012), Determination of glucosamine and carisoprodol in pharmaceutical formulations by LC with pre-column derivatization and UV detection; Journal of chromatographic science, 50(4); 307-315.
15. Zenon K. and Kinga B; (1998), Simultaneous determination of salicylic acid and acetylsalicylic acid in aspirin delayed release tablets formulation by second derivative UV spectrophotometry; Jour. of Pharmaceutical Biomedical Analysis; 18(4-5); 871-875.