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# A PRECISE RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF DIAZEPAM AND PROPRANOLOL HCL IN TABLET DOSAGE FORM

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## ABSTRACT

In the simultaneous estimation of Diazepam and Propranolol HCl by RP-HPLC method development, the optimized chromatographic condition was selected by taking a mobile phase combination of mixed phosphate buffer (0.02M potassium dihydrogen orthophosphate and 0.003M dipotassium orthophosphate pH adjusted to 3.0 with ortho-phosphoric acid) and acetonitrile in a ratio of 40:60 v/v. BDS Hypersil (250x4.6 I.D, 5µ) was selected as the column. The flow rate was selected as 1.0mL/ min. The UV- detection wavelength was taken as 222nm, by using this detection was carried out. The method development was carried out by external standard method. By the use of the above chromatographic conditions, the peaks are found to be symmetrical, with good resolution. The retention times of Diazepam and Propranolol HCl was found to be 2.031 and 5.597 min. The run time was found to be short and the peaks are eluting with good resolution. The linearity studies of Diazepan and Propranolol HCl were found to be in the range of 2-12µg/mL and 16-96µg/mL respectively. The slope, intercept and correlation coefficient of Diazepam and Propranolol HCl were 26.01, 11.87, 0.999 and 4.328, 11.54, 0.999 respectively. The linearity data suggests that the linearity was within the Beer Lamberts limit.

## KEYWORDS

Diazepam, Propranolol HCl spectrophotometric, LC-MS, Liquid chromatography, HPTLC and Capillary electrophoresis.

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## INTRODUCTION

HPLC is a form of column chromatography that pumps at high pressure a sample (analyte) dissolved in a solvent (mobile phase) through a column with an immobilized chromatographic packing material (stationary phase). Separation of compounds in a sample can be accomplished via an isocratic elution, where the composition of the mobile phase remains

constant, or via a gradient elution, where the mobile phase composition is changed over the course of the separation toward conditions favoring analyte dissociation from the stationary phase a UV-absorbance detector. Selection of the appropriate detector and monitoring wavelengths is essential for optimizing the sensitivity of HPLC detection. The detector generates a signal correlating to the quantity of analyte emerging from the column, which is then transferred to and recorded by an HPLC control computer program, with the data available for subsequent analysis. Analytical chemistry is can be described as the area of chemistry responsible for characterizing the composition of matter, both qualitatively (what is present) and quantitatively (how much is present). The analytical chemistry is "the science of inventing and applying the concepts, principles, and strategies for measuring the characteristics of chemical systems and species." Analytical chemists typically operate at the extreme edges of analysis and improving the ability of all chemists to make appropriate measurements on smaller samples, on more complex samples, on shorter time scales. Throughout its history, analytical chemistry has provided many of the tools and methods as fostering multidisciplinary research like, medicinal chemistry, clinical chemistry, toxicology, forensic chemistry, geochemistry, and environmental chemistry.

## MATERIAL AND METHODS

### Drug Samples

Diazepam and Propranolol hydrochloride raw materials were provided as gift samples from Chandra labs, Hyderabad.

### Formulation Used

Dizipax tablets (Altiusunimarck pharma) containing 2.5mg of Diazepam and 20mg Propranolol hydrochloride was purchased from the local mark.

### Reference Standards

### Diazepam

The percentage purity is 100.97%.

### Propranolol hydrochloride

The percentage purity of the raw material is 99.95%.

### Instruments

The instruments used for the work includes the following.

HPLC Prominence Shimadzu.

Pump : LC-20 AT VP series.

Injector : Rheodyne.

Column : BDS Hypersil C18 (250mm x4.5mm id, 5µ)

Double beam UV-Visible Spectrophotometer Evaluation E 100.

Digital balance - Sartorious (0.1mg - 205mg)

pH meter -Elico.

Vacuum pump -Gelman Science.

## METHOD DEVELOPMENT

### Solubility

Drug solubility plays an important role in the development of a method. According to the literature review, Diazepam and Propranolol HCl are shows good solubility in water and acetonitrile. So as per the literature reviews the solubility of drugs were checked with various ratios of acetonitrile and buffers. From the above a mixed phosphate buffer and Acetonitrile in the ratio of 40:60% v/v were selected for the present work.

### Selection of Chromatographic Method

Selection of a method depends upon the nature of the drug molecule, drug solubility, and molecular weight of the moiety. So as the drugs Diazepam and Propranolol HCl were polar in nature reverse phase chromatographic technique was selected for the work.

### Selection of wavelength

Selection of wavelength is the most important criteria before starting a method development. In setting the conditions for development of the assay method, the choice of detecting wavelength was determined by scanning the absorption spectrum for Diazepam and Propranolol HCl. A solution of 8µg/mL concentration of Diazepam and Propranolol HCl was scanned in the range of 200-400nm against blank. Both drugs showed maximum absorption at

222nm, so this wavelength was taken for further analysis.

## METHOD DEVELOPMENT TRIALS

**Result**

The retention times of Diazepam and propranolol HCl was found to be 6.1, 8.5. But the asymmetry was found to be 3.576.

**Result**

The retention time of Diazepam and Propranolol HCl were 8.5 and 3.5. But the elution time was prolonged.

**Result**

The Propranolol HCl peak doesn't elute properly.

**Result**

Peaks were broad and the asymmetry was found to be 3.1.

**Result**

The run time of both the drugs were increased.

**Result**

The retention times of Diazepam and Propranolol HCl were found to be 2.03 and 5.60 respectively. The asymmetry was 1.57, 1.31.

**Conclusion**

Out of the 6 trials which were performed, the 6$^{th}$ trial was selected as the optimized condition for the method development, because the retention time was good, the asymmetry was found to be within the limits, the resolution was good.

## RESULTS AND DISCUSSION

A precise reverse phase chromatography for the simultaneous estimation of Diazepam and Propranolol HCl was developed.

A solution containing 8µg/mL of Diazepam and Propranoplol HCl was prepared and scanned in the UV - region. Both the drugs show a maximum absorbance at 222nm. Therefore the wavelength 222nm was selected as the detection wave length. The spectrum was shown in the Figure No.7.

For getting optimized chromatographic conditions various trials have been performed by changing the mobile phase, pH, and the composition of the mobile phase. The trials were performed by taking mobile phase compositions as 50:50, 45:55, 55:45and the flow rates were also altered by taking 1.0mL and 1.2mL. The trials which were performed shows prolonged retention time, and asymmetry that is more than two, and decrease in resolution. Finally a mobile phase composition of acetonitrile and mixed phosphate buffer in a composition of 60:40 with a pH of 3.0, flow rate of 1.0mL per minute shows good resolution, asymmetry, lesser run time. So this chromatographic condition was selected as an optimized chromatographic condition. The chromatograms regarding the trials were shown in the Figure No.1-6. The retention times of Diazepam and Propranolol HCl was found to be 2.003 and 5.603 respectively. By using the optimized chromatographic conditions a stock solution of Diazepam and Propranolol HCl was prepared by using the mobile phase (Buffer and acetonitrile in 40:60 % v/v pH 3.0). And various concentrations were prepared in the range of 16 - 96µg/mL for Propranolol HCl and 2 - 12µg/mL for Diazepam, and injected to the chromatographic system. The slope, intercept and correlation coefficient were calculated. The slope, intercept, correlation coefficient of Diazepam was found to be 4.328, 11.54 and 0.999 and for Propranolol HCl it was found to be 26.01, 11.87, 0.999. Calibration curve was plotted by taking response factor vs. concentration. The calibration curve shows that the linear response was obtained over the range of concentrations used. The range demonstrates that the method is linear outside the limit of expected use.

**Table No.1: Reagents and chemicals used**

| S.No | Chemical Name | Grade | Make |
|---|---|---|---|
| 1 | Methanol | HPLC | Rankem |
| 2 | Acetonitrile | HPLC | Rankem |
| 3 | Water | HPLC | Millipore |
| 4 | Ortho phosphoric acid | Analytical | Rankem |
| 5 | Potassium dihydrogen phosphate | Analytical | Rankem |
| 6 | Di potassium hydrogen phosphate | Analytical | Rankem |

**Table No.2: Trial -1**

| S.No | Mobile phase | Column | Flow rate |
|---|---|---|---|
| 1 | ACN: Water(50:50) | Ymc ODS C18 (150x4.6mm) | 1mL/min |

**Table No.3: Trial -2**

| S.No | Mobile phase | Column | Flow rate |
|---|---|---|---|
| 1 | 0.1M$KH_2PO_4$:CAN (50:50) | BDS Hypersil C18 (250x4.6mm) | 1mL/min |

**Table No.4: Trial -3**

| S.No | Mobile phase | Column | Flow rate |
|---|---|---|---|
| 1 | ACN: Water(50:50) | BDS Hypersil C18(150x4.6mm) | 1mL/min |

**Table No.5: Trial - 4**

| S.No | Mobile phase | Column | Flow rate |
|---|---|---|---|
| 1 | Phosphate buffer: CAN (55:45) | BDS Hypersil C18 (250x4.6mm) | 1.2mL/min |

**Table No.6: Trial -5**

| S.No | Mobile phase | Column | Flow rate |
|---|---|---|---|
| 1 | Mixed phosphate buffer: CAN (55:45) | BDS Hypersil C18(150x4.6mm) | 1mL/min |

**Table No.7: Trial - 6**

| S.No | Mobile phase | Column | Flow rate |
|---|---|---|---|
| 1 | ACN : mixed phosphate buffer(60:40) pH3.0 | BDS Hypersil C18 (250x4.6mm) | 1mL/min |

**Assay Results**

| Area | S.No | Peak Area of Diazepam | Peak Area of Propranolol HCl |
|---|---|---|---|
| **Standard** | 1 | 272.616 | 362.235 |
| | 2 | 272.081 | 362.081 |
| | 3 | 274.957 | 364.609 |
| | **Avg** | 273.390 | 362.975 |
| **Sample** | 1 | 280.732 | 365.827 |
| | 2 | 278.604 | 366.399 |
| | **Avg** | 279.668 | 366.113 |
| | **% Assay** | 99.95 | 100.97 |

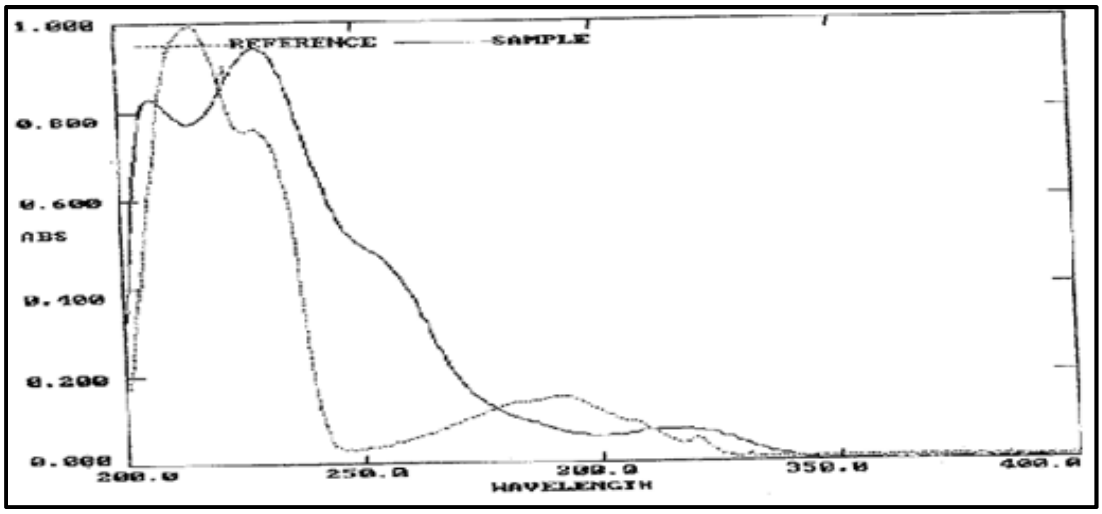
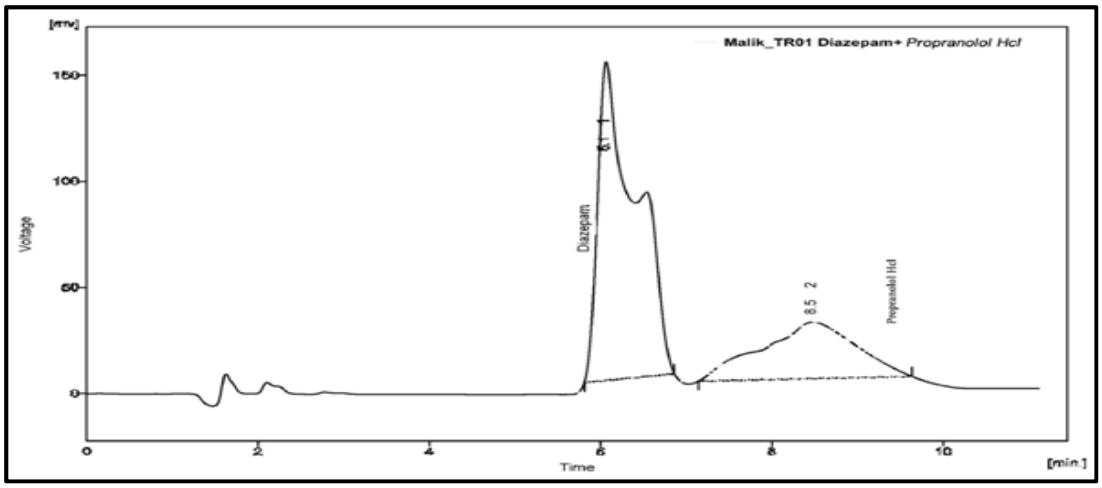
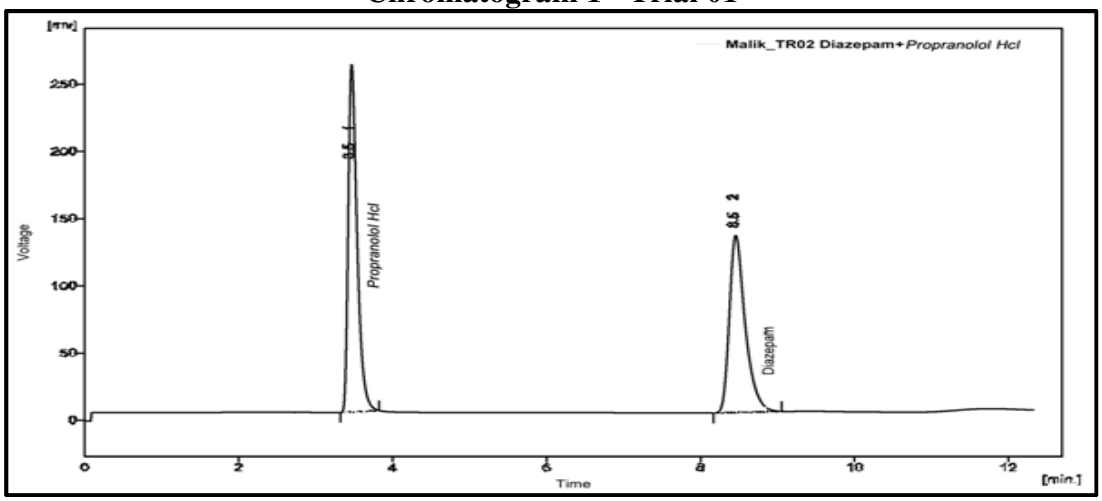



**Figure No.1: UV - Spectrum of Diazepam and Propranolol HCl**

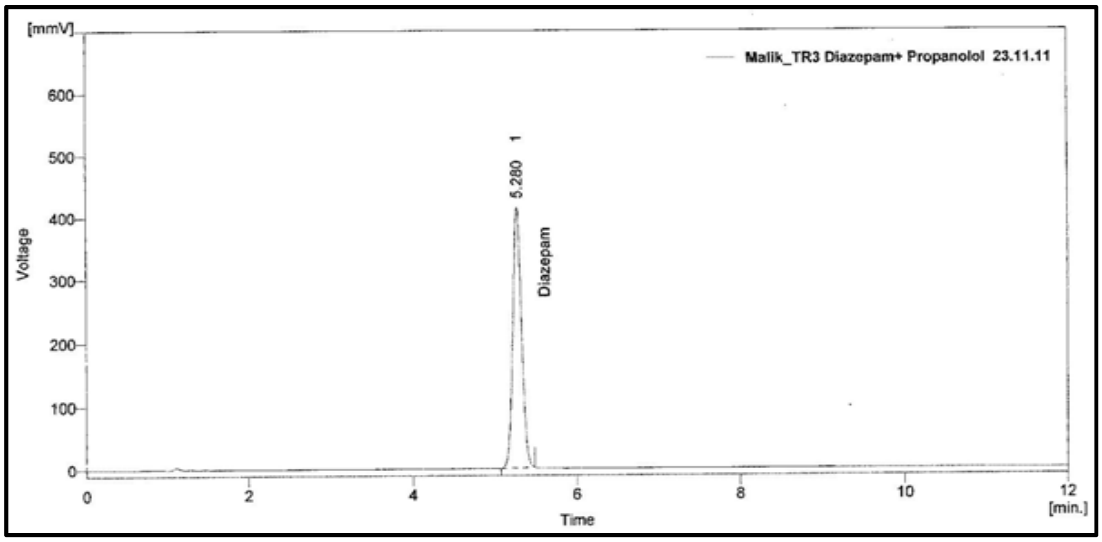
**CHROMATOGRAMS**



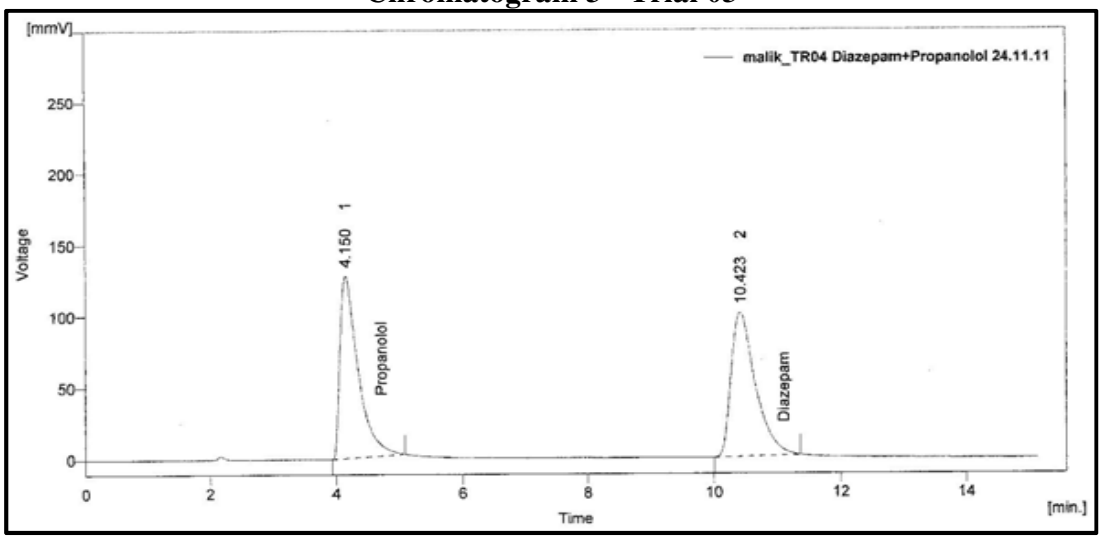

**Chromatogram 1 - Trial 01**



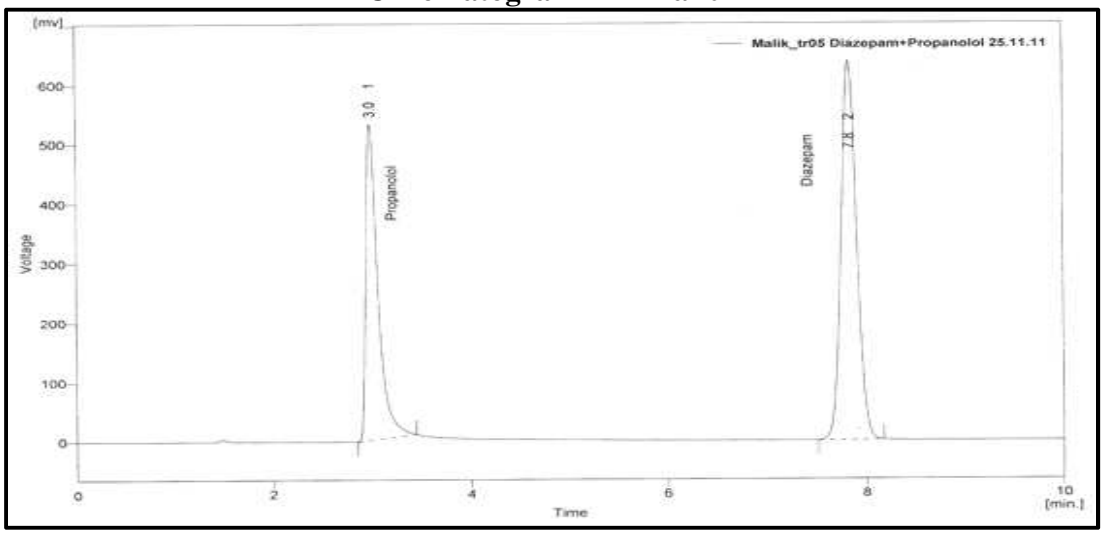

**Chromatogram 2 - Trial 02**



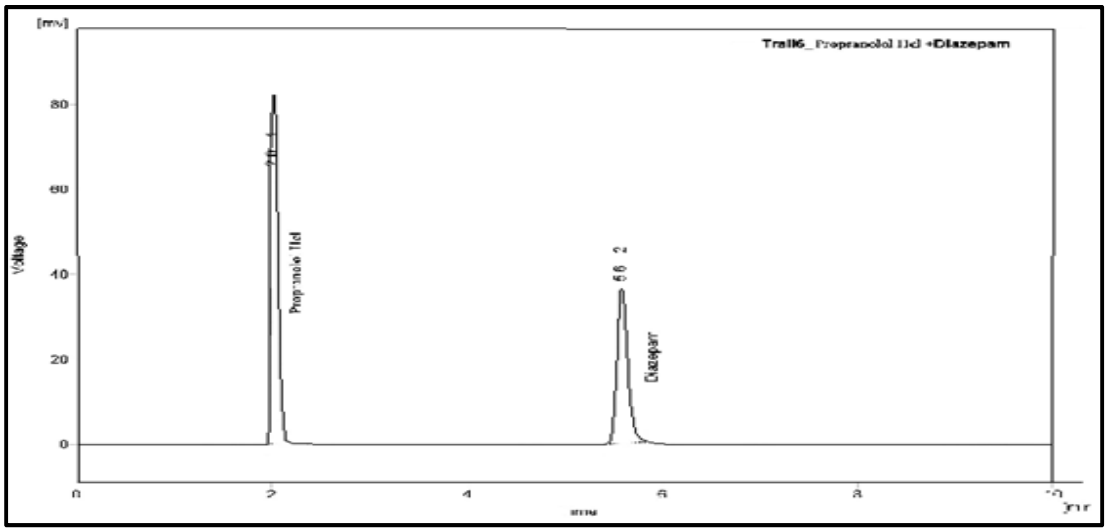

**Chromatogram 3 - Trial 03**



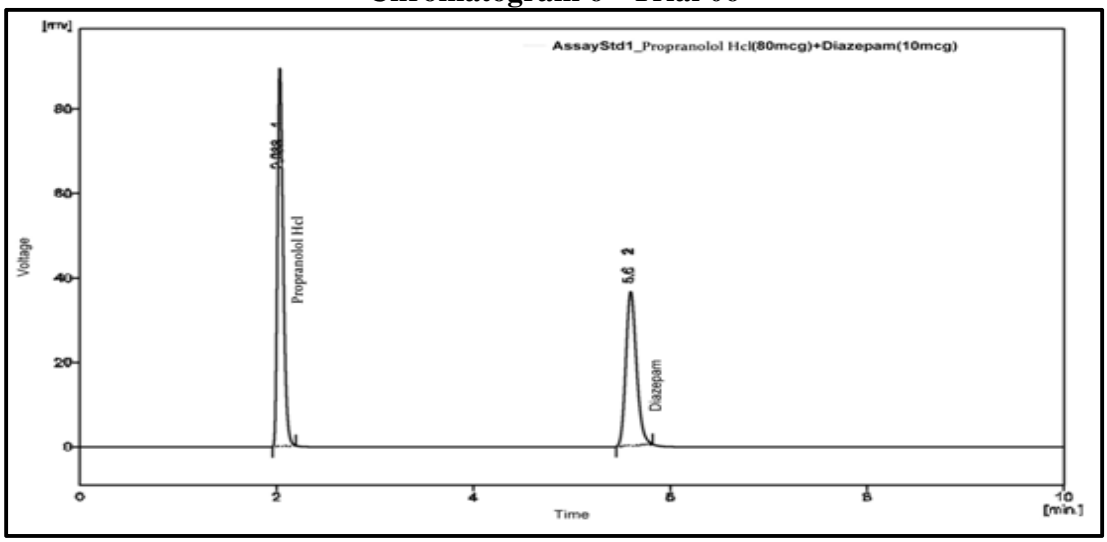

**Chromatogram 4 - Trial 04**



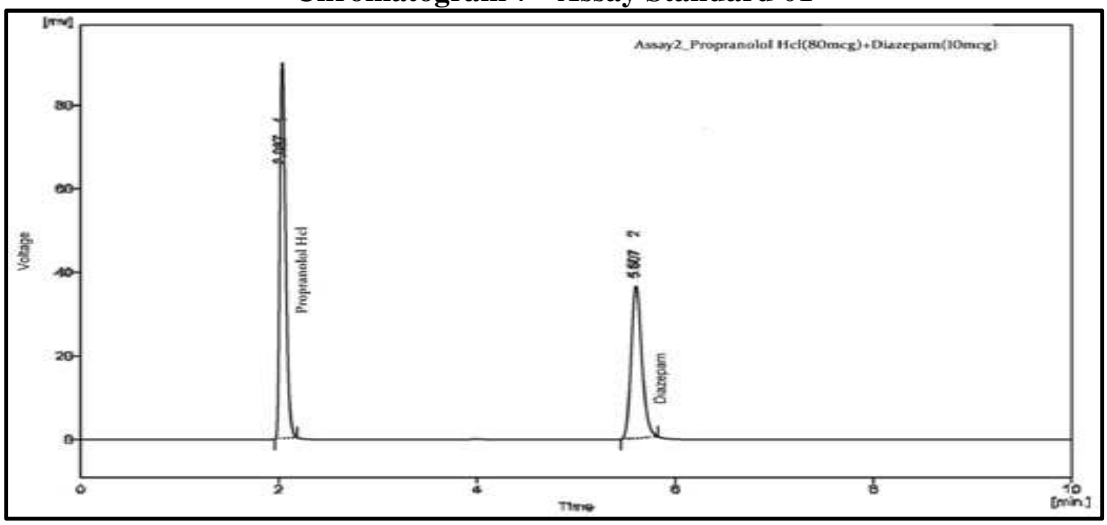

**Chromatogram 5 - Trial 05**



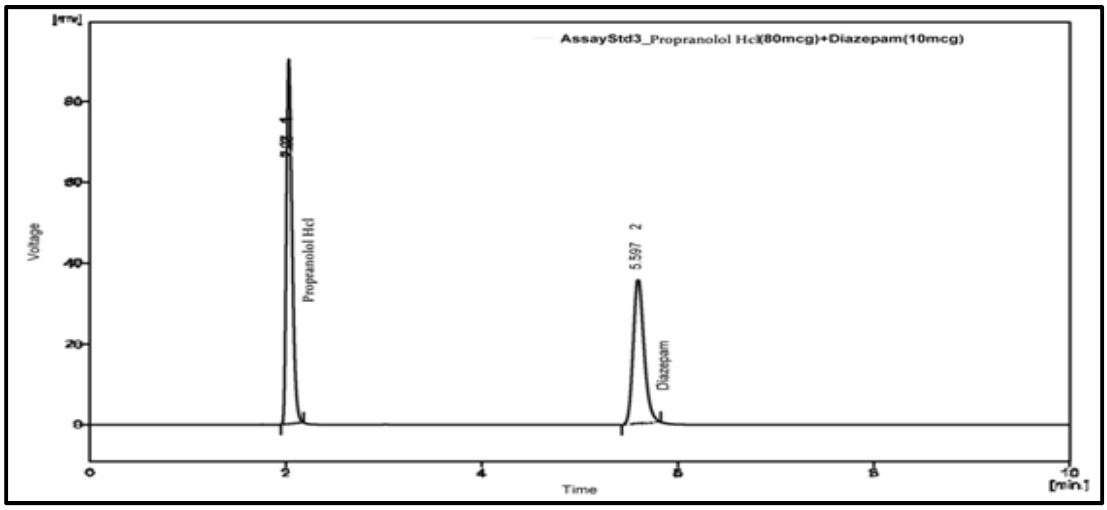

**Chromatogram 6 - Trial 06**



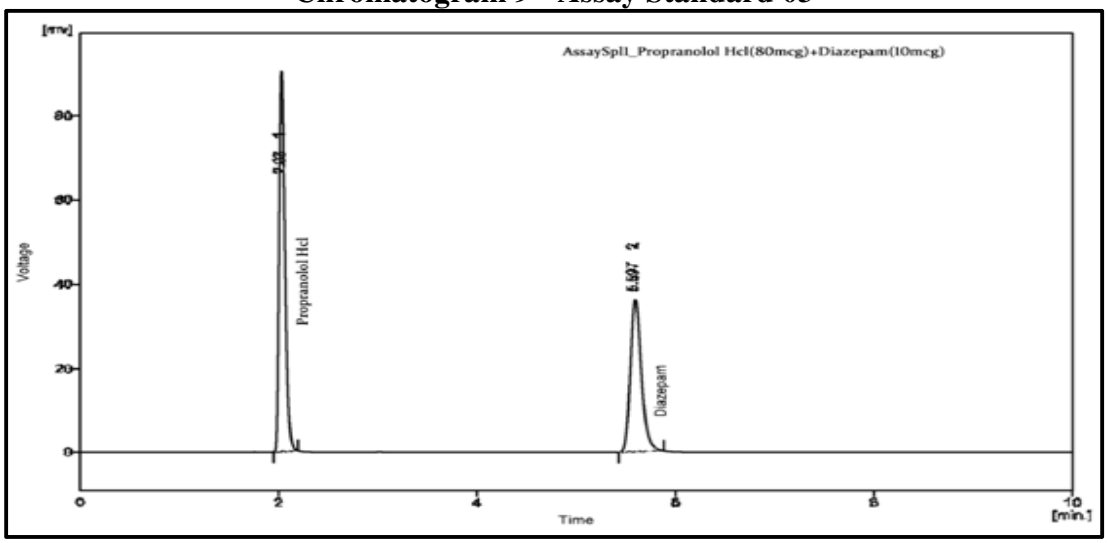

**Chromatogram 7 - Assay Standard 01**



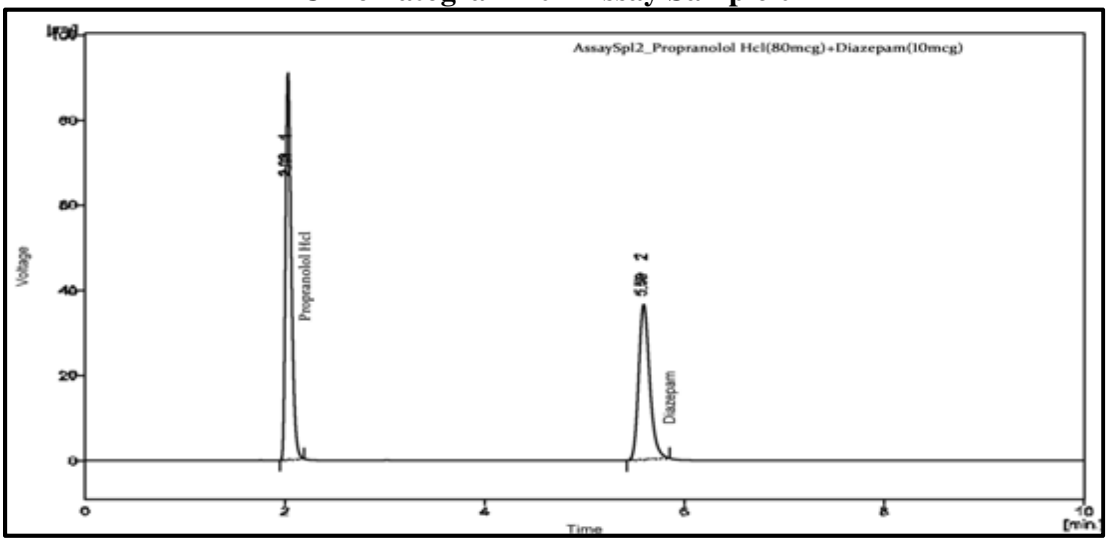

**Chromatogram 8 - Assay Standard 02**




**Chromatogram 9 - Assay Standard 03**




**Chromatogram 10 - Assay Sample 01**




**Chromatogram 11 - Assay Sample 02**

## SUMMARY AND CONCLUSION

From the literature review, it was confirmed that few methods had been reported for the estimation of Diazepam and Propranolol HCl in combined dosage forms, individually and with other drugs. Various works such as the spectrophotometric, LC-MS, liquid chromatography, HPTLC, capillary electrophoresis and bio analytical studies was reported

In the simultaneous estimation of Diazepam and Propranolol HCl by RP-HPLC method development, the optimized chromatographic condition was selected by taking a mobile phase combination of mixed phosphate buffer (0.02M potassium dihydrogen ortho phosphate and 0.003M dipotassium ortho phosphate pH adjusted to 3.0 with ortho phosphoric acid) and acetonitrile in a ratio of 40:60 v/v. BDS Hypersil (250x4.6 I.D, 5µ) was selected as the column. The flow rate was selected as 1.0mL/ min. The UV- detection wavelength was taken as 222nm, by using this detection was carried out. The method development was carried out by external standard method. By the use of the above chromatographic conditions, the peaks are found to be symmetrical, with good resolution. The retention times of Diazepam and Propranolol HCl was found to be 2.031 and 5.597 min. The run time was found to be short and the peaks are eluting with good resolution.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

## BIBLIOGRAPHY

1. Amod S. Patil, Atul A. Shirkhedkar, Sanjay J. Surana, Prajakta S. Nawale. Q-Absorbance and multicomponent UV spectrophotometric methods for simultaneous estimation of propranolol hydrochloride and flunarizine dihydrochloride in capsules, *Der Pharma Chemica,* 3(3), 2011, 404-408.
2. Anonymous. ICH Harmonized tripartile guidelines, text on validation of analytical procedure, Text and methodology, *Q2A, Geneva,* 1994, 1-5.
3. Anonymous United States Pharmacopoeia. 23/NF, 18, *United States of Pharmacopoeia Convention, Inc, Rock Wille, MD,* 1063, 1961, 1988 1990.
4. Ashutoshkar. Pharmaceutical analysis, *CBS Publishers and Distributors Pvt. Ltd,* 2007, 11.
5. Becket A H, Stenlake J B. Practical pharmaceutical chemistry. Part - II, *CBS Publishers and Distributors, New Delhi,* 4th Edition, 2007, 85, 86, 92.
6. Chung Chow Chan, Herman Lam, Lee Y C, XUE-Ming Zhang. Analytical method validation and instrument performance verification, A *John Wiley and Sons, Inc, Publications,* 2004, 16-22.
7. David G. Watson. A text book for pharmacy students and pharmaceutical chemist, *Churchill and Livingstone Publishers Limited,* 1999, 195, 201.
8. David Harvey. Modern analytical chemistry, *Published by Mc Graw Hill Higher Education, A Division of The Mc Graw-Hill Companies,* 2000, 2-6.
9. Kealey D, Haines P G. Instant notes on analytical chemistry, *Bios Scientific Publishers Limited,* 2002, 1-5.
10. Douglas A. Skoog, Donald M. West. Principles of instrumental Analysis, *Saunders Golden Sun Burst Series,* 2nd Edition, 2007, 667, 693-705.
11. Eleanor I. Miller, Fiona M. Wylie, John S. Oliver. Simultaneous detection and quantification of amphetamines, diazepam and its metabolites, cocaine and its metabolites, and opiates in hair by LC–ESI-MS–MS using a single extraction method, *Journal of Analytical Toxicology,* 8(2), 2008, 457-469.

12. Georze Z. Tsogas, Dimitrios V. Stergiou, Athanasios G. Vlessidis, Nicholas P. Evmiridis. Development of a sensitive flow injection-chemiluminescence detection method for the indirect determination of propranolol, *Analytica Chemical Acta,* 541(205), 2005, 151-157.
13. Girija Bhavar, Chatpalliwar V A. Quantitative analysis of propranolol hydrochloride by high performance thin layer chromatography, *Indian Journal of Pharmaceutical Sciences,* 70(3), 2008, 395-398.
14. Gurdeep R Chatwal, Sham K Anand. Instrumental methods of chemical analysis, *Himalaya Publishing House, Mumbai,* 5th Edition, 2006, 2.566, 2.624.

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