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Research

Validate Precise Rp- Hplc Method For The Simultaneous Estimation Of Diazepam And Propranolol Hcl

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Abstract

High Performance Liquid Chromatography (HPLC) is a very important analytical tool for the assessments of drug product. HPLC techniques should be able to separate, identify and quantify various medicinal products and related detoriating materials that can form on storage or manufacturing or may be incorporated during the synthesis. Validation is the process of establishing the performance and limitations of any technique and identification of various products which may change their characteristics. This article discusses the strategies and the issues pertinent to modelling of HPLC method development and validation.

Keywords: HPLC, Impurities, Method development, Validation

INTRODUCTION

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).

- More appropriate description of 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, and on species present at lower concentrations.
- Throughout its history, analytical chemistry has provided many of the tools and methods necessary for research in the other four traditional areas of chemistry, as well as fostering multidisciplinary research

like, medicinal chemistry, clinical chemistry, toxicology, forensic chemistry, geochemistry, and environmental chemistry.

- Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter.
- Qualitative analysis is the identification of elements, species and or compounds present in a sample.
- Quantitative analysis is the determination of the absolute or relative amounts of elements, species or compounds present in a sample.
- Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (Functional groups).
- An element, species or compound that is the subject of analysis is known as an analyte.
- The remainder of the material or sample of which the analytes forms a part is known as the matrix.

1.1 The Analytical Perspective (David Harvey *et al.*, 2001)

Each field of chemistry brings a unique perspective to the study of chemistry many analytical chemists describe this perspective as an analytical approach to solving problems. Although there are probably as many descriptions of the analytical approach as there are analytical chemists, it is convenient for our purposes to treat it as a five-step process.

1. Identifying and defining the problem.
2. Designing the experimental procedure.
3. Conduct an experiment, and gather data.
4. Analyze the experimental data.
5. To propose a solution to the problem.

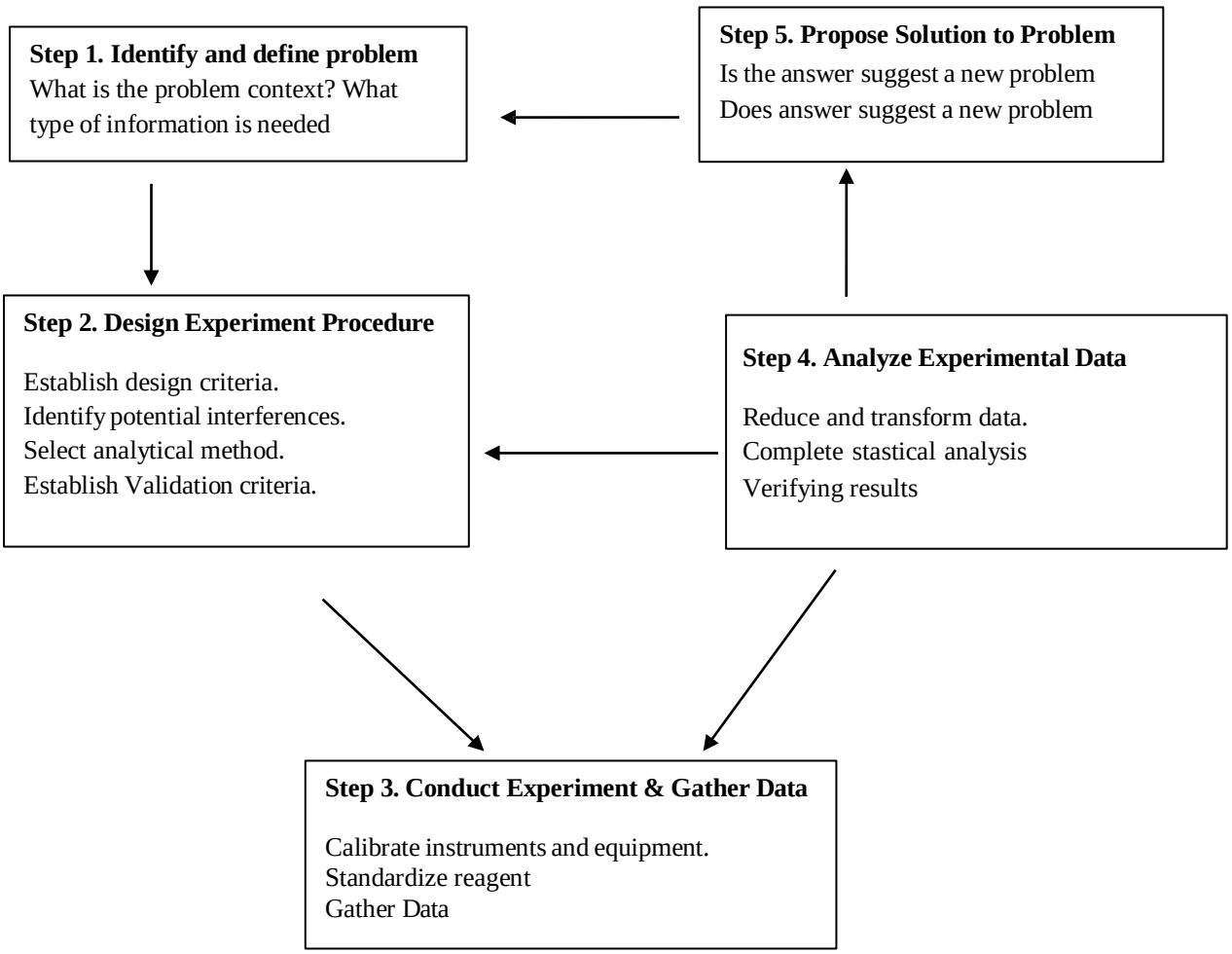


Fig 1: Flow diagram of Analytical Perspective

Scope and Applications (D. Kealey, *et al.*, 2002)

Analytical data are required in a wide range of disciplines and situations that include not only include chemistry but most other sciences, from biology to zoology, also the arts, such as painting and sculpture, and archaeology. Space exploration and clinical diagnosis are two quite unique areas in which analytical data is vital. Important areas of application include the following.

a) Quality control (QC)

In many manufacturing industries, the chemical composition of raw materials, intermediates and finished products needs to be monitored to ensure satisfactory quality and consistency. Analytical chemistry is vital in the field of quality control.

b) Clinical and biological studies

The levels of important nutrients, including trace elements such as sodium, potassium, calcium and zinc, naturally produced chemicals, such as cholesterol, sugars, urea, and administered drugs in the body fluids of patients are required to monitor. These biological studies are complex in nature. Analytical chemistry is one of the important tool in clinical and biological studies.

c) Fundamental and applied research

The chemical composition and structure of materials used during the research programs in numerous disciplines can be of significance. Where new drugs or materials with potential commercial value are synthesized, a complete chemical characterization may be required involving considerable analytical work. Various studies like spectroscopic determinations, drug solubility, and other data regarding the newly developing drugs can be estimated.

d) Monitoring and control of pollutants

The presence of toxic heavy metals such as, lead, cadmium and mercury, organic chemicals includes polychlorinated biphenyls and detergents and vehicle exhaust gases like oxides of carbon, nitrogen and sulphur, and hydrocarbons in the environment are health hazards and necessary action should taken. These can monitored by sensitive and accurate methods of analysis.

e) Qualitative analysis

Qualitative analysis is the determination of the quality of the substance present in a sample. It Tests on the sample under specified and controlled conditions. Qualitative analysis is the tests on reference materials for comparison and interpretation of the tests

f) Quantitative analysis

Preparation of standards containing known amounts of the analyte or of pure reagents to be reacted with the analyte. Calibration of instruments to determine the responses to the standards under controlled conditions. Measurement of the instrumental response for each sample under the same conditions as for the standards.

g) Sample pre-treatment or conditioning.

Sample pre treatment plays an important role before going for analysis. Conversion of the sample into a form suitable for detecting or measuring the level of the analyte by the selected technique and method. This can be performed by dissolving it, converting the analyte into a specific chemical form or separating the analytes from other components of the sample that could interfere with detection or quantitative measurements.

Instrumentation of HPLC (Douglas A. Skoog *et al*., 2007**)** HPLC instrumentation consists of the following components.

- Solvent Reservoir (HPLC solvent reservoir systems)
- Pumps
- PreGuard Column
- Sample injection system
- Columns
- Detector
- Recorder and integrators

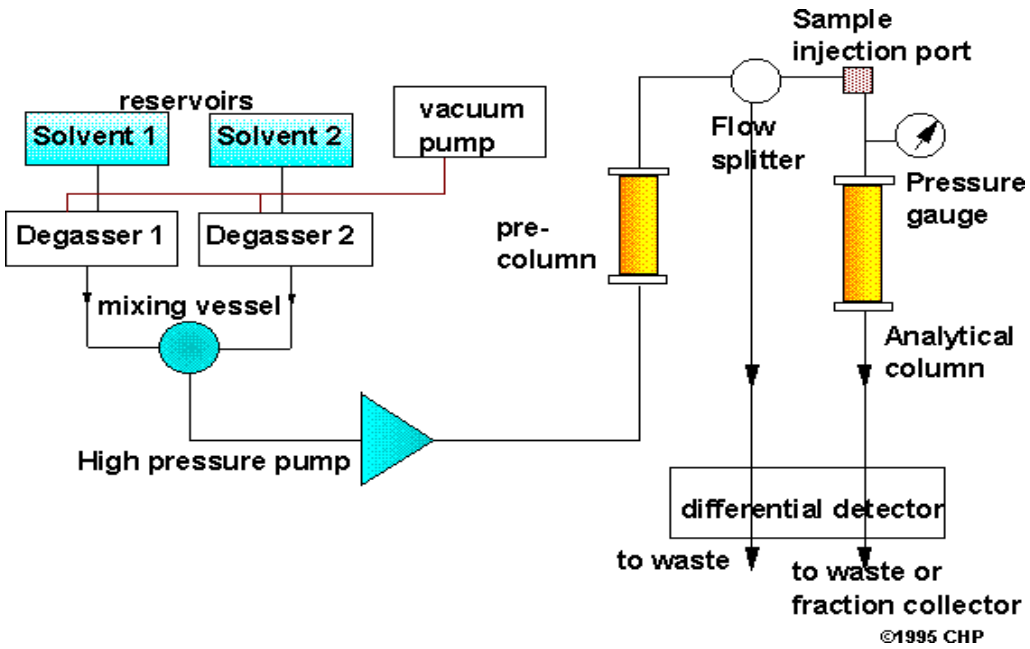


Fig 2: Block Diagram of HPLC Instrument

MATERIALS AND METHODS

MATERIALS

Drug Samples

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

Formulation Used

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

Reagents and Chemicals Used

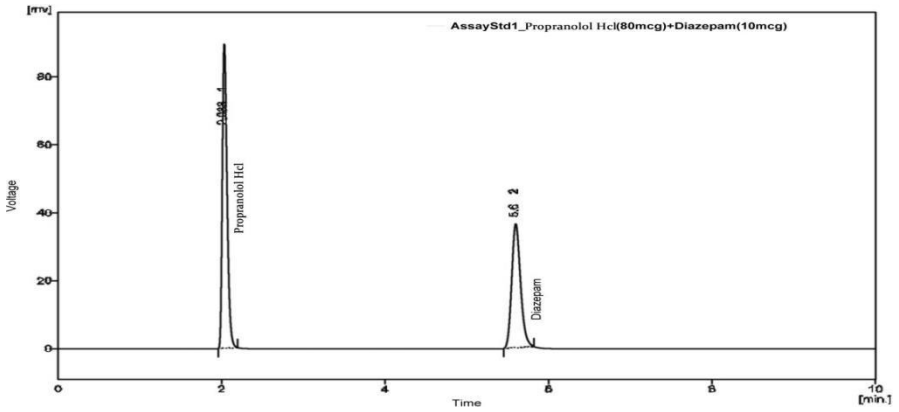
Table 1: Reagents and Chemicals Used

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

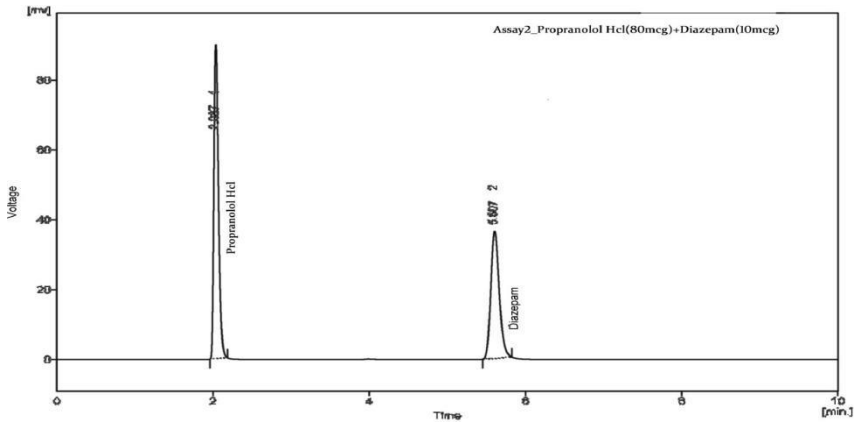
METHOD VALIDATION

Performing validation is responsible for interpreting guidance into acceptable practices. The method was validated according to the ICH guidelines. Various parameters included in the method validation include the following.

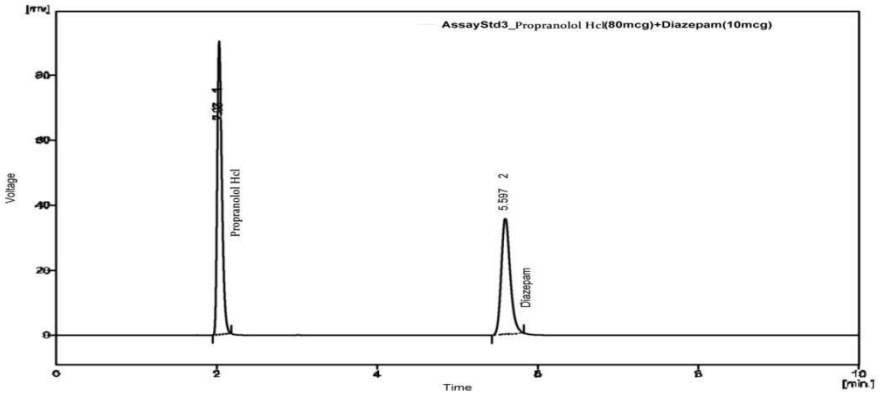
- Specificity
- Accuracy
- Precision
- Linearity
- Robustness
- Ruggedness
- Limit of detection



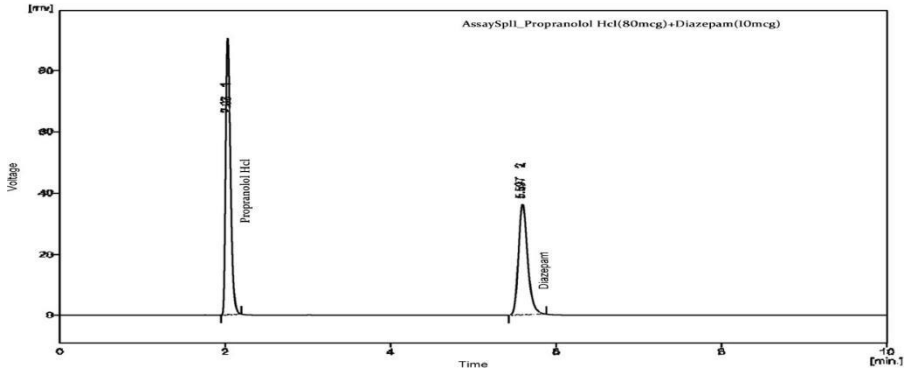
Chromatogram 7 – Assay Standard 01



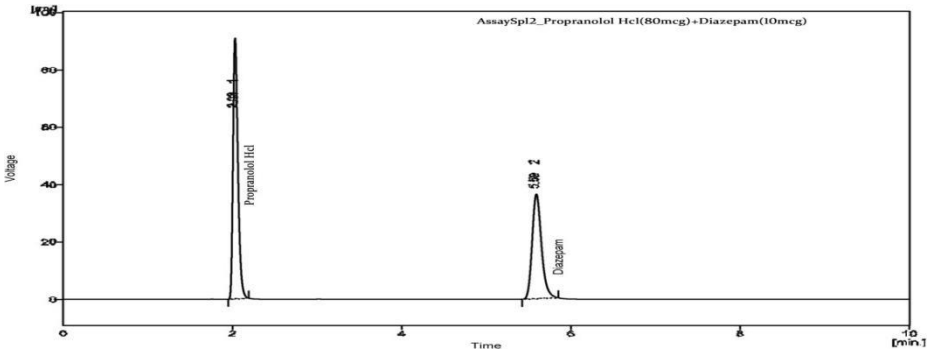
Chromatogram 8 – Assay Standard 02



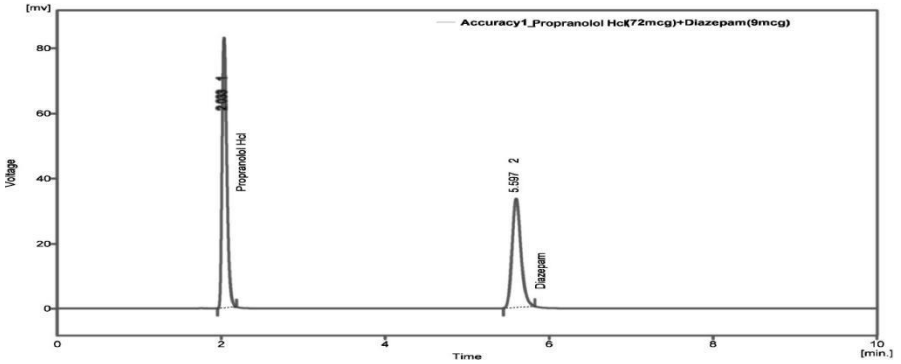
Chromatogram 9– Assay Standard 03



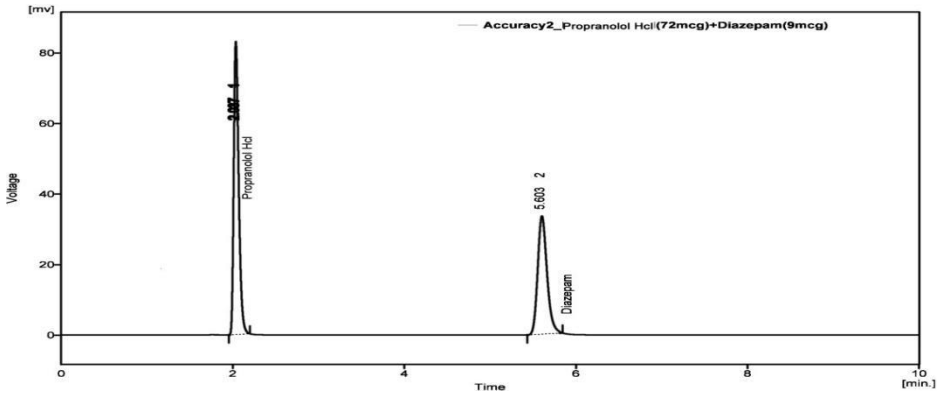
Chromatogram 10 – Assay Sample 01



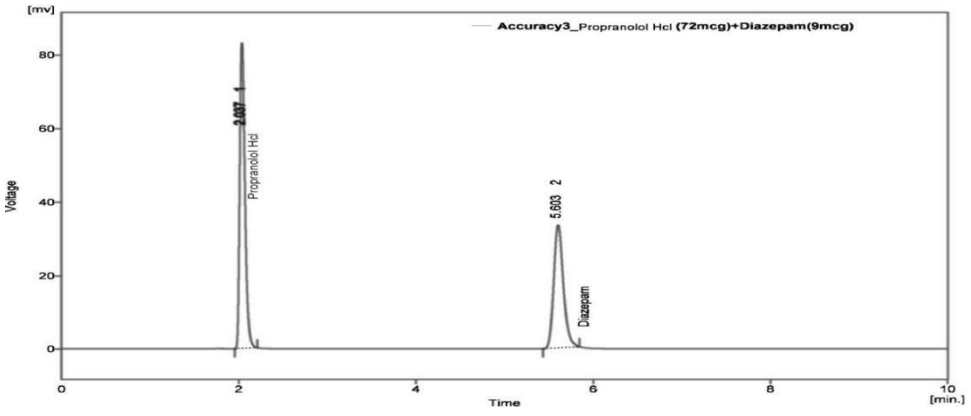
Chromatogram 11 – Assay Sample 02



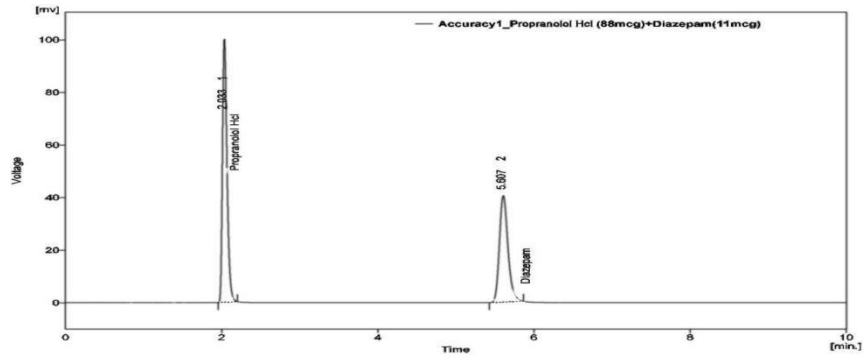
Chromatogram 12 – Accuracy 01



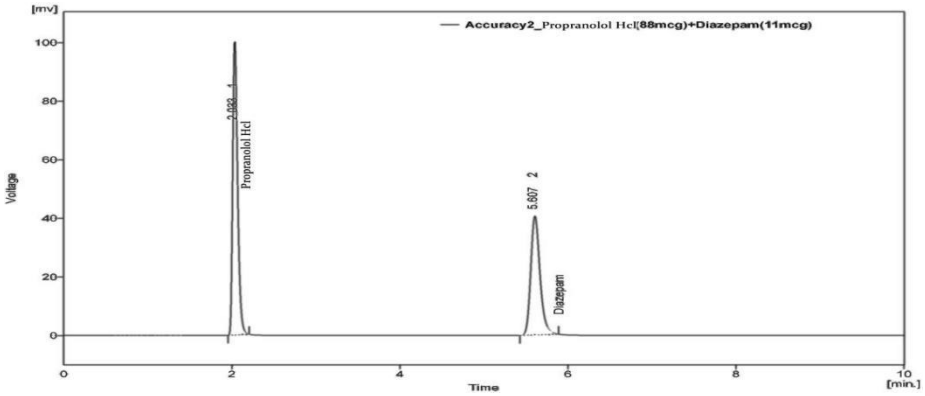
Chromatogram 13 – Accuracy 02 (Propranolol HCl 72 µg + Diazepam 9 µg/mL)



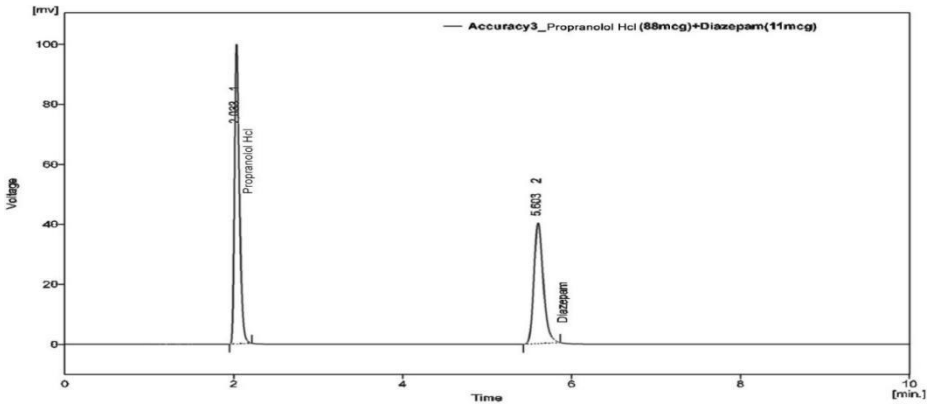
Chromatogram 14 – Accuracy 03 (Propranolol HCl 72 µg + Diazepam 9 µg/mL)



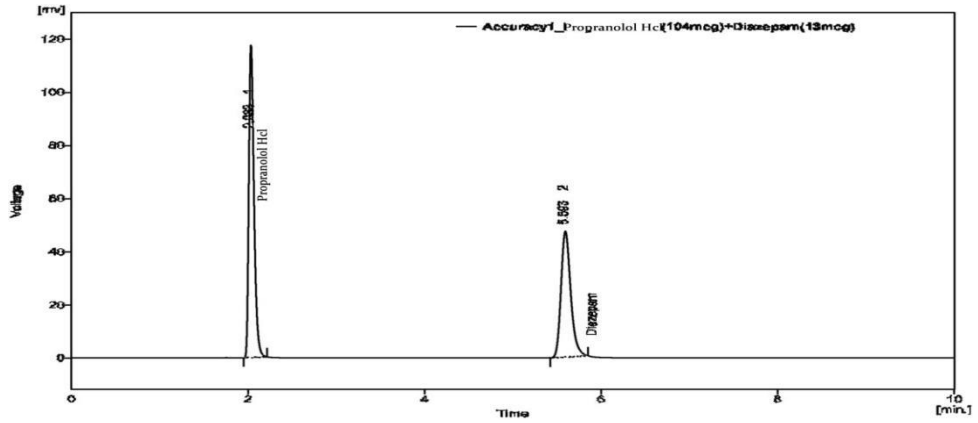
Chromatogram 15 – Accuracy 01 (Propranolol HCl 88 µg + Diazepam 11 µg/mL)



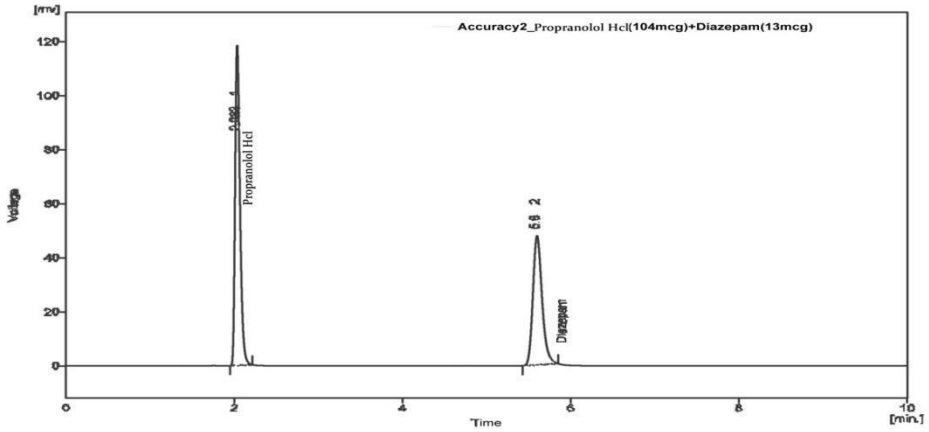
Chromatogram 16 – Accuracy 02 (Propranolol HCl 88 µg + Diazepam 11 µg/mL)



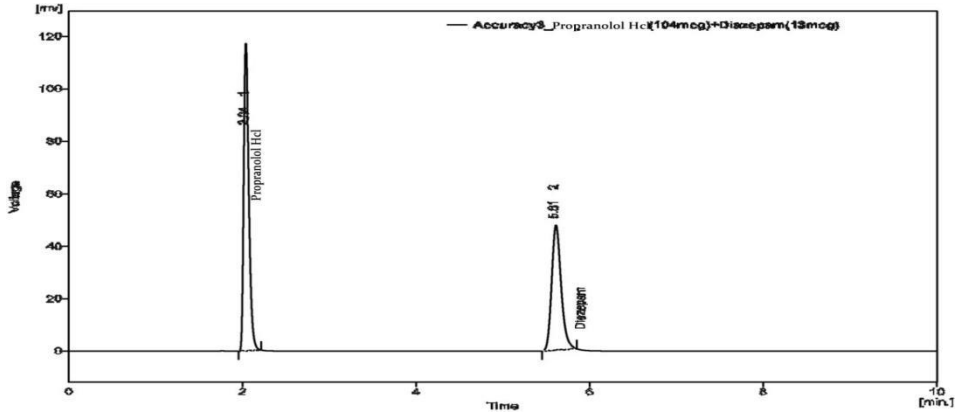
Chromatogram 17 – Accuracy 03 (Propranolol HCl 88 µg + Diazepam 11 µg/mL)



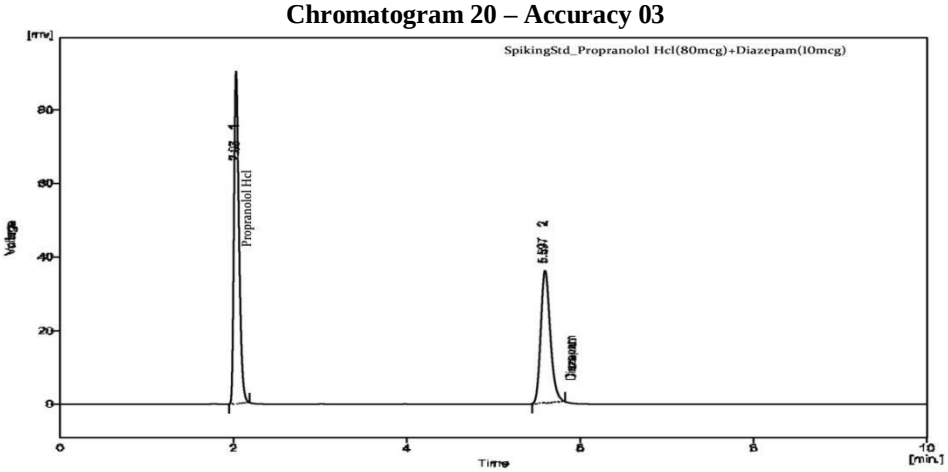
Chromatogram 18 - Accuracy 01 (Propranolol HCl 104 µg + Diazepam 13 µg/mL)



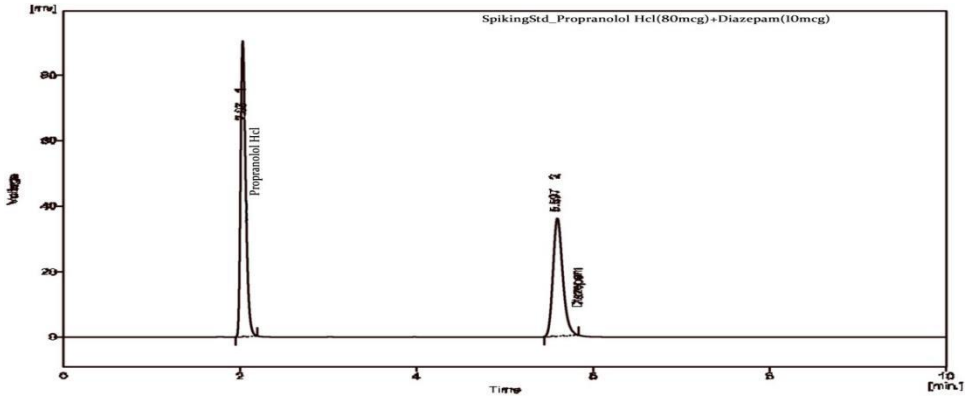
Chromatogram 19 - Accuracy 02 (Propranolol HCl 104 µg + Diazepam 13 µg/mL)



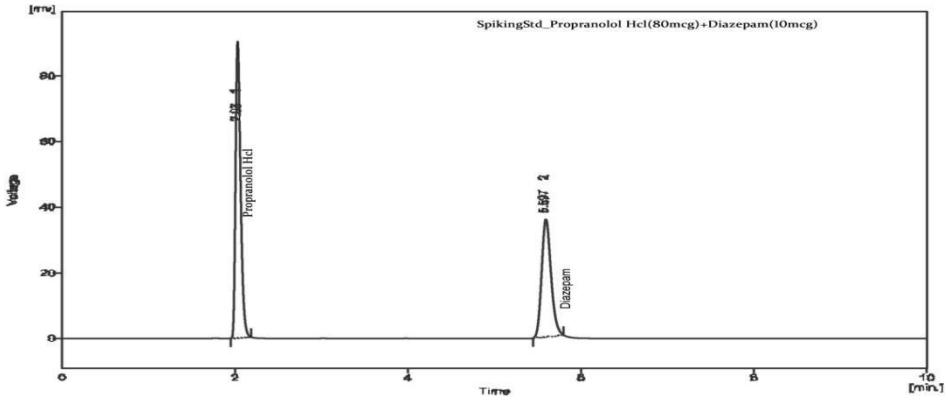
Chromatogram 20 – Accuracy 03



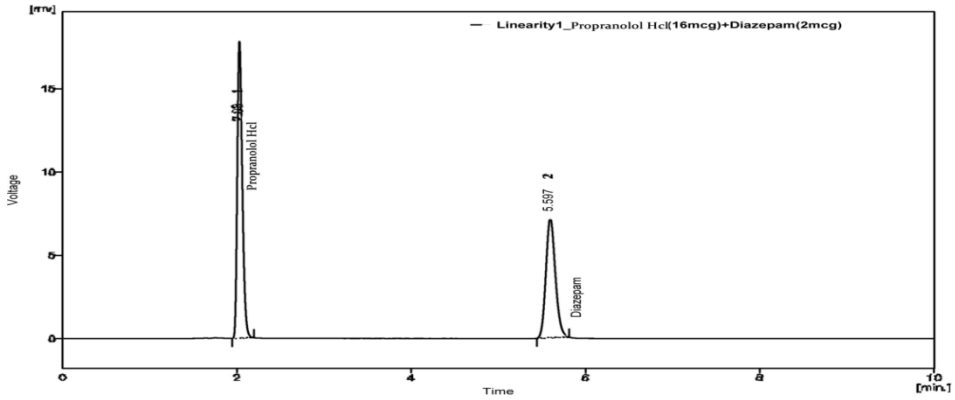
Chromatogram 21 – Spiking Standard 01 (Propranolol HCl 80 µg + Diazepam 10 µg/mL)



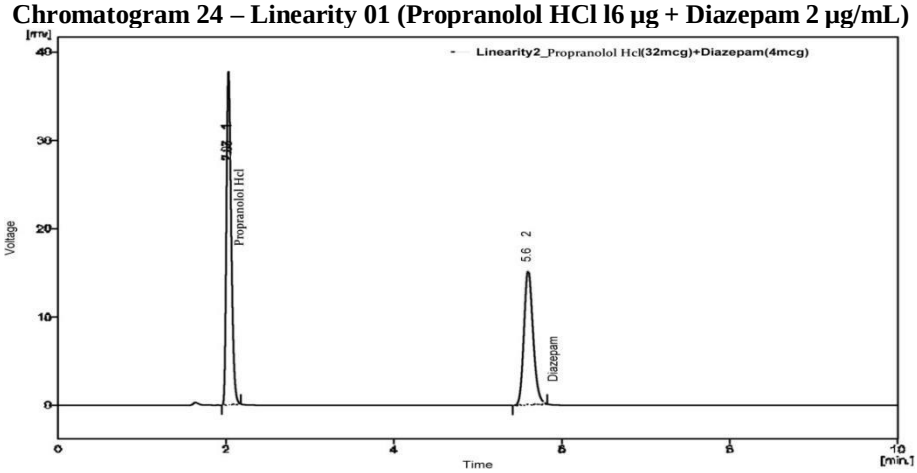
Chromatogram 22 – Spiking Standard 02



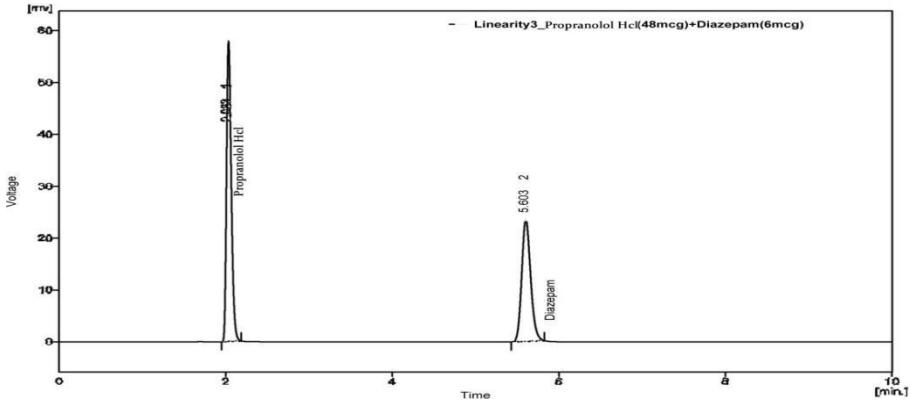
Chromatogram 23 – Spiking Standard 03 (Propranolol HCl 80 µg + Diazepam 10 µg/mL)



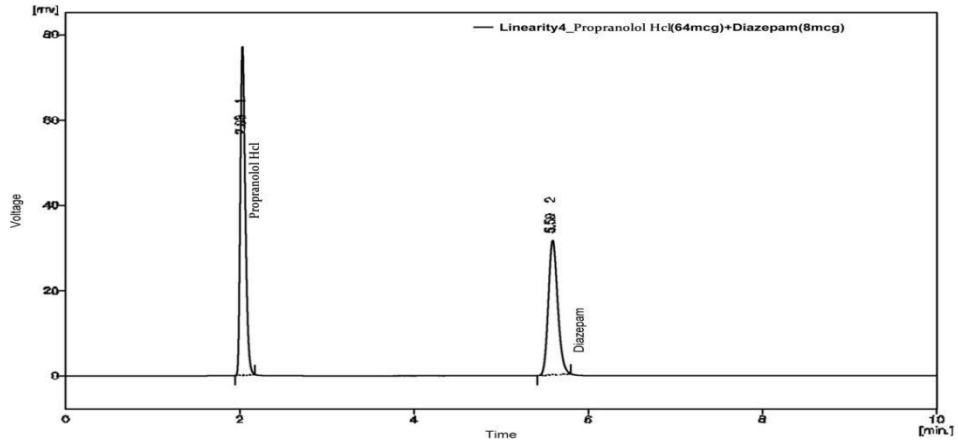
Chromatogram 24 – Linearity 01 (Propranolol HCl l6 µg + Diazepam 2 µg/mL)



Chromatogram 25 – Linearity 02 (Propranolol HCl 32 µg + Diazepam 4 µg/mL)



Chromatogram 26 – Linearity 03



Chromatogram 27 - (Propranolol HCl 48 µg + Diazepam 6 µg/mL)

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 222 nm. Therefore the wavelength 222 nm was selected as the detection wave length. The spectrum was shown in the figure 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, 55:45, and the flow rates were also altered by taking 1.0 mL and 1.2 mL. 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 1.0 mL 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 1 - 6. The retention times of Diazepam and Propranolol HCl was found to be 2.003 and 5.603 respectively.

Estimation

Simultaneous estimation of Diazepam and Propranolol HCl in tablet dosage form was developed by reverse phase HPLC. The chromatograms were recorded by using the sample and the standard solutions. From these standard and sample solutions, the assay was performed and the percentage was estimated both in individual drugs and in tablet formulations. The method developed for the simultaneous estimation of Diazepam and Propranolol HCl by RP – HPLC was found to be accurate, precise, simple, linear, rapid and rapid.

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 1.0 mL/ min. The UV- detection wavelength was taken as 222 nm, 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 2-12 µg/mL and 16-96 µg/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.

Precision studies were performed with respect to system precision and intermediate precision. The %RSD was found to be within the acceptable limit. From this it was conclude that the method was found to be precise enough. The robustness was performed by bringing deliberate changes to the method condition and the method was found to be robust. The recovery of the Diazepam and Propranolol HCl was found to be within the acceptable limit. It indicates that the method was accurate. The present works of RP-HPLC method development have various advantages

- The run time for the elution was found to be shorter.
- The preparation of sample and standards are simple and does not consume more time.
- The instrument used for the method development is commonly available and it is cost effective.

From the above mentioned parameters it was concluded that the method development of Diazepam and Propranolol HCl was found to be simple, rugged, specific, linear, precise, accurate and reliable.

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*.2011, 3(3): 404-408.
2. Anonymous. 1994. ICH Harmonized Tripartile Guidelines, Text on Validation of Analytical procedure: Text and Methodology.Q2A. Geneva. pp 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: pp 11.
5. Becket AH, Stenlake JB. Practical Pharmaceutical Chemistry. Part –II. 4th edition. CBS Publishers and Distributors: New Delhi. 2007: pp 85, 86, 92.
6. Chung Chow Chan, Herman Lam, Y.C Lee, XUE-Ming Zhang. Analytical Method Validation and Instrument Performance Verification. A John Wiley and Sons, Inc., Publications.2004: pp 16-22.
7. David G. Watson, A Text Book For Pharmacy Students and Pharmaceutical Chemist.
8. Churchill and Livingstone Publishers Limited. 1999: pp 195, 201David Harvey, Modern Analytical Chemistry. Published by Mc Graw Hill Higher Education, A Division of The Mc Graw-Hill companies. 2000: pp 2 – 6.
9. D. Kealey, P.J. Haines. Instant Notes on Analytical Chemistry. Bios Scientific Publishers Limited. 2002: pp 1-5.
10. Douglas A. Skoog, Donald M. West. Principles of instrumental Analysis. 2nd edition. Saunders Golden Sun Burst Series.2007: pp 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.* 2008, 8(2):457-469.