CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.13899820

https://www.iajps.com/volumes/volume11-october-2024/09-issue-10-october-24/

Available online at: http://www.iajps.com *Research Article*

QUANTITATIVE ESTIMATION OF HYOSCINE BUTYL BROMIDE AND PARACETAMOL IN TABLET DOSAGE FORMS BY RP--HPLC METHOD

S.Shyamala*, Mrs.R.Madhulika,R.Mounika,Dr.K.Shravankumar

[1]Department Of Pharmaceutical Analysis, Samskruti College Of Pharmacy, Ghatkesar, Telangana. 501301.

Article Received: August 2024 Accepted: September 2024 Published: October 2024

Abstract:

A new, simple, rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validation of Hyoscine butyl bromide and Paracetamol in its pure form as well as in combined marketed formulation. Chromatography was carried out on a X bridge C18 (4.6mm×150mm) 5µm particle size column using a mixture of ACN: Methanol (70:30% v/v) as the mobile phase at a flow rate of 1.2ml/min, the detection was carried out at 260nm. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The method produce linear responses in the concentration range of 20-60mg/ml of Hyoscine butyl bromide and 10-30mg/ml of Paracetamol. The inter-day and intra-day precisions were found to be within limits. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: *Hyoscine butyl bromide and Paracetamol, RP-HPLC, Validation, Accuracy, Precision.*

Corresponding author:

S.Shyamala,

Department of Pharmaceutical Analysis,
Samskruti college of pharmacy,
Ghatkesar, Telangana.
Email Id- shyamumudiraj46@gmail.com

QR code

Please cite this article in press **S.Shyamala** *et al.,* ***Quantitative Estimation Of Hyoscine Butyl Bromide And Paracetamol In Tablet Dosage Forms By RP--HPLC Method.,****Indo Am. J. P. Sci, 2024; 11 (10).*

INTRODUCTION:

Pharmaceutical analysis comprises those procedures necessary to determine "identity, strength, quality and purity of the drug substances and drug products. Pharmaceutical analyst plays a major role in all quality controlling divisions of industry. Analytical chemistry involves separating, identifying, and determining the relative amounts of components in a sample matrix. The number of new drugs is constantly growing. This requires new methods for controlling the quality. Modern pharmaceutical analysis must need the following requirements [1].

1. The analysis should take a minimal time.
2. The accuracy of the analysis should meet the demands of the Pharmacopoeia.
3. The analysis should be performed with a minimal cost.
4. Precision and selectivity of the selected method should be good.

Typical Instrumental Techniques [2,3]:

The methods of estimation of drugs are divided into physical, chemical, physicochemical and biological ones of them, physical and physicochemical methods are used mostly. Physical methods of analysis involve the studying of the physical properties of a substance. They include determination of the solubility, transparency or degree of turbidity, colour density or specific gravity (for liquids), moisture content, melting, freezing and boiling points. Physicochemical methods are used to study the physical phenomenon that occurs as a result of chemical reactions. Among the physicochemical methods are optical refractometry, polarimetry, emission and fluorescent methods of analysis, photometry including photocolorimetry, spectrophotometry, nephelometry and turbidometry, electrochemical (potentiometry, amperometry, coulometer, polarography) and chromatography (column, paper, thin layer, gas, high performance liquid) methods are generally preferable.

Methods involving nuclear reactions such as nuclear magnetic resonance (NMR) and paramagnetic resonance (PMR) are becoming more popular. The combination of mass spectroscopy with gas chromatography is one of the most powerful tools available. The chemical methods include the gravimetric and volumetric procedures, which are based on complex formation, acid-base and precipitation and redox reactions. Titrations in non-aqueous media and complexometry have been widely used in pharmaceutical analysis whenever the existing amounts are in milligram level and the interference is negligible. The methods (LC-MS,[4] HPLC, GLC, NMR and Mass Spectroscopy) of choice for assay involve sophisticated equipment that are very costly and pose problems of maintenance. Hence, they are not in the reach of most laboratories and small-scale industries, which produce bulk drugs and pharmaceutical formulations.

The visible Spectrophotometric methods which fall in the wavelength region 400-800 nm and fluorimetric methods (may fall in UV & Visible regions) are very simple, cheap and easy to carry out estimations of drugs in bulk form and their formulations. The limitations of many colorimetric or fluorimetric methods of analysis lie in the chemical reactions upon which the procedures are based rather than the instruments available. Many of the reactions involve colour or fluorescence of a drug are quite selective or can be rendered selective through the introduction of masking agents, control of PH, use of solvent extraction technique, adjustment of oxidation states or by prior removal of interfering ingredients with the aid of chromatographic separation.

1. This is preferably followed by general methodology for UV-Visible and HPLC method developments.
2. Followed by literature of drugs used in Analysis

Introduction to hplc:

Russian botanist Tswett invented chromatography as a separation technique. He describes in detail the separation of pigments, the colour substances by filtration through column, followed by developments with pure solvents.

High-performance liquid chromatography (HPLC) [5] is the fastest growing analytical technique for analysis of drugs. Its simplicity, high specificity and wide range of sensitivity make it ideal for the analysis of many drugs in both dosage forms and biological fluids.

According to IUPAC, chromatography [6] is a physical method of separation in which components will be separated or distributed between stationary and mobile phases. The importance of chromatography is increasing rapidly in pharmaceutical analysis for the exact differentiation, selective identification and quantitative determination of structurally closely related compounds. Another important field of application of chromatographic methods is the purity testing of final products and the intermediates. The reasons for the popularity of the method is its sensitivity, its ready adaptability to accurate quantitative determinations, its suitability for separating non-volatile species or thermally fragile ones and its wide spread applicability to substances that are of prime interest to the industry. Sensitive detectors have transformed liquid column

chromatography into high speed, efficient, accurate and highly resolved method of separation.

The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

- Speed (many analysis can be accomplished in 20 min or less)
- Greater sensitivity (various detectors can be employed)
- Improved resolution (wide variety of stationary phases)
- Reusable columns (expensive columns but can be used for many analysis)
- Ideal for the substances of low viscosity
- Easy sample recovery, handling and maintenance.
- Instrumentation leads itself to automation and quantification (less time and less labour)
- Precise and reproducible
- Integrator itself does calculations.

Instrumentation [7]:

- The essential parts of the High Performance Liquid Chromatography are:

1) Solvent reservoir and Treatment system
2) Mobile phase
3) Pump system[17]
4) Sample Injection System
5) Column
6) Detector

Hplc Method Development: [9,10]

A good method development strategy should require only as many experimental runs as are necessary to achieved the desired final result. Finally, method development should be as simple as possible, and it should allow the use of sophisticated tools such as computer modelling. During initial method development, a set of initial conditions (detector, column, mobile phase) is selected to obtains the first "scouting" chromatograms of the sample. In most cases, these are based on reversed-phase separation on a C_{18} column with UV detection. The important factors, which are to be taken into account to obtains reliable quantitative analysis, are

- Careful sampling and sample preparation.
- Appropriate choice of the column.
- Choice of the operating conditions to obtains the adequate resolution of the mixture.
- Reliable performance of the recording and data handling systems.
- Suitable integration/peak height measurement technique.
- The mode of calculation best suited for the purpose.
- Validation of the developed method.

Before beginning method development, it is need to review what is known about the sample in order to define the method goals.

Sample And Analyte Information:

This information is useful for the selection of appropriate sample preparation procedures as well as the initial detection and chromatographic modes. If critical data are not available (e.g., pKa, solubility), separate studies should be initiated as soon as possible. The chemical structure of the analyst furnishes data on molecular weight and the nature of the functional groups. Particular attention should be directed to acidic, basic, aromatic, or reactive functional groups from which estimates of pKa, solubility, chromophoric, or stability data can be inferred. If sufficient purified reference material is available, solubility studies of the analyst in common solvents such as water, alcohol, ether, and hexane should be conducted. Toxicity data and Material Safety Data Sheets (MSDS), COA including spectral data (MS, NMR, IR, and UV) should be ascertained. The chemical composition of the sample can provides valuable clues for the best choice of initial conditions for an HPLC separation.

Selection of chromatographic mode [11]:

Reversed-phase chromatography (RPC), the most common mode for small organic molecules. Note that ionisable compounds (acids and bases) are often separate by RPC with buffered mobile phases (to keep the analyte in a non-ionized state) or with ion-pairing reagents. In reverse phase mode, the mobile phase is comparatively more polar than the stationary phase. For the separation of polar or moderately polar compounds, the most preferred mode is reverse phase. The nature of the analyte is the primary factor in the selection of the mode of separation.

Sample preparation [12]:

Samples comes in various forms

- Solutions ready for injection
- Solutions that require dilution, buffering, addition of an internal standard or other volumetric manipulation
- Solids must be dissolved or extracted

- Samples that require pre-treatment to remove interferences and/or protect the column or equipment from damage.

Most Samples for HPLC analysis require weighing and /or volumetric dilution before injection. Best results are often obtained when the composition of the sample solvent is close to that of the mobile phase since this minimizes baseline upset and other problems. Some sample require a particles separation (pre-treatment) prior to HPLC, because of need to remove interferences, concentrate sample analytes or eliminate "column killers".

Choice of the column:
Selection of the column is the first and the most important step in method development. The appropriate choice of separation column indicates three different approaches.

- Selection of separation.
- The particle size and nature of the column packing.
- The physical parameters of the column i.e. the length and the diameter.

Some of the important parameters considered while selecting chromatographic columns are

- Length and diameter of the column
- Packing material
- Shape of the particles
- Size of the particles
- % of Carbon loading
- Pore volume
- Surface area
- End capping

Selection of column temperature:
Always carry out chromatographic separations at ambient temperature. The increase in column temperature generally will result in reduction of asymmetry and peak retention. The column temperature between 30°c – 80°c is shall be adopted if necessary. If a column temperature above 80°c is required, packing material which can be with stand to that temperature was preferable.

Selection of solvent delivery system:
Chromatographic separation with isocratic elution i.e. all constituents of the mobile phase is mixed and pumped together as a single solvent, is always preferable however, gradient elution is powerful tool in achieving separation between closely eluting compounds or compounds having widely differing in polarities.

The important future of gradient elution is that the polarity and ionic strength of the mobile phase can be changed during run. The mobile phases are introduced into column by two different ways, low pressure and high pressure gradient systems. Low pressure gradient can be adopted when not more than 80% of organic phase is to be pumped or vice-versa. While optimizing gradient elution especially low viscous solvents like acetonitrile and phosphate buffer, it is recommended to mix about 10% aqueous portion preferably the same buffer used in mobile phase to avoid pumping problems.

The gradient programme which is rugged for organic phase up to 10% variation and up to 0.2 ml variation in flow rate was selected.

Selection of mobile phase:
The primary objective in selection and optimization of mobile phase is to achieve optimum separation of all impurities and degradants from each other and from analyte peak.

In liquid chromatography, the solute retention is governed by the solute distribution factor, which reflects the different interactions of the solute-stationary phase, solute-mobile phase, and mobile phase-stationary phase. For a given stationary phase, the nature and the composition of which has to be judiciously selected in order to get appropriate and required solute retention. The mobile phase has to be adapted in terms of elution strength (solute retention) and solvent selectivity (solute separation). Solvent polarity is the key word in chromatographic separations since a polar mobile phase will give rise to low solute retention in normal phase and high solute retention in reverse phase LC. The selectivity will be particularly altered if the buffer pH is close to the pKa of the analytes. The following are the parameters, which shall be taken into consideration while selecting and optimizing the mobile phase.

- Buffer and its strength
- pH of the buffer or pH of the mobile phase
- Mobile phase composition

MATERIALS AND METHODS:

Hyoscine butyl bromide -Procured from Mylon, Provided by Sura Pharma labs, Paracetamol-Procured from Mylon, Provided by Sura Pharma labs, Water and Methanol for HPLC-LICHROSOLV (MERCK).

Hplc method development:
Mobile Phase Optimization:
Initially the mobile phase tried was Water: Methanol and ACN: Methanol with varying proportions. Finally,

the mobile phase was optimized to ACN, Methanol in proportion 70:30 v/v respectively.

Optimization of Column:

The method was performed with various columns like C18 column ODS column, Zodiac column, and Xterra C18 column. X bridge C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions:

Instrument used : Waters HPLC with auto sampler and PDA detector 996 model.
Column : X bridge C18 (4.6×150mm) 5 µ
Mobile phase : Methanol: ACN (30:70v/v)
Flow rate : 1.2 ml per min
Wavelength : 260 nm
Injection volume : 10 µl
Run time : 8 min.

Preparation of buffer and mobile phase:

Preparation of mobile phase:

Accurately measured 300 ml (30%) of Methanol and 700 ml of ACN (70%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION:

Trial 7 (Optimized):

Mobile phase : Methanol: ACN (30:70v/v)
Column : X bridge (4.6×150mm, 5 µ)
Flow rate : 1.2 ml/min
Wavelength : 260 nm
Column temp : Ambient
Injection Volume : 10 µl
Run time : 8 min

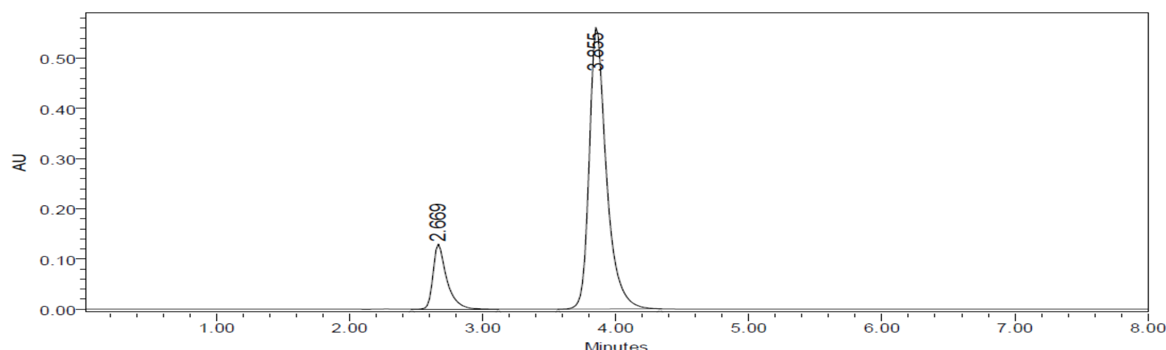

Figure7.7: Chromatogram for trail 7

Table7.7: - peak results for trail 7

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Hyoscine butyl bromide	2.669	986575	128673		1.5	3553.0
2	Paracetamol	3.855	5365217	562208	1.8	1.4	4675.8

Observation:

This trial shows improper separation sample peaks, baseline and show very less plate count in the chromatogram. So it's required more trials to obtain good peaks.

From the above chromatogram it was observed that the Hyoscine butyl bromide and Paracetamol peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Retention time of Hyoscine butyl bromide – 2.669min

Retention time of Paracetamol–3.855min

System suitability:

Table: Results of system suitability parameters for Hyoscine butyl bromide and Paracetamol

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Hyoscine butyl bromide	2.669	979868	129659		1.7	3855
2	Paracetamol	3.855	5356472	587453	1.9	1.9	4797

Acceptance criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.

Assay (Standard):

Table: Showing assay standard results

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Hyoscine butyl bromide	2.669	986588	127855		1.6	3554	1
2	Paracetamol	3.855	5387452	561415	1.7	1.4	4655	1
3	Hyoscine butyl bromide	2.669	987825	126986		1.5	3572	2
4	Paracetamol	3.855	5378476	568952	1.7	1.4	4636	2
5	Hyoscine butyl bromide	2.654	986542	127895		1.5	3842	3
6	Paracetamol	3.849	5369876	568476	1.7	1.4	4685	3

Assay (Sample):

Table7.9: Showing assay sample results

S.No.	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Hyoscine butyl bromide	2.669	988627	127855		1.6	3562	1
2	Paracetamol	3.855	5387548	568542	1.7	1.4	4875	1
3	Hyoscine butyl bromide	2.651	989686	127842		1.5	3659	2
4	Paracetamol	3.849	5392436	563525	1.7	1.4	4642	2
5	Hyoscine butyl bromide	2.621	989875	127857		1.5	3855	3
6	Paracetamol	3.840	5389855	565413	1.7	1.4	4366	3

Table-7.10: Showing assay results

S.No	Name of compound	%purity
1	Hyoscine butyl bromide	99 %
2	Paracetamol	100%

The retention time of Hyoscine butyl bromide and Paracetamol was found to be 2.669min and 3.855mins respectively. The % purity of Hyoscine butyl bromide and Paracetamol in pharmaceutical dosage form was found to be 99% and 100% respectively.

Precision:

Table: Results of method precision for Hyoscine butyl bromide:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Hyoscine butyl bromide	2.669	986858	128232	3654	1.5
2	Hyoscine butyl bromide	2.659	987855	129853	3542	1.5
3	Hyoscine butyl bromide	2.671	985473	128146	3636	1.5
4	Hyoscine butyl bromide	2.669	986588	129612	3596	1.5
5	Hyoscine butyl bromide	2.669	985212	128320	3699	1.5
Mean			986397.2			
Std. Dev			1076.193			
% RSD			0.109103			

Table: Results of method precession for Paracetamol:

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Paracetamol	3.855	5378558	565622	4676	1.4	1.7
2	Paracetamol	3.842	5386232	564588	4697	1.4	1.7
3	Paracetamol	3.850	5385412	563652	4685	1.4	1.7
4	Paracetamol	3.845	5369875	563545	4764	1.4	1.7
5	Paracetamol	3.855	5389746	578548	4955	1.4	1.7
Mean			5381965				
Std. Dev			7880.495				
% RSD			0.146424				

Acceptance criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision/Ruggedness:

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

Table-: Results of Intermediate precision for Hyoscine butyl bromide

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Hyoscine butyl bromide	2.669	978986	128875	3687	1.5
2	Hyoscine butyl bromide	2.529	975687	128366	3655	1.5
3	Hyoscine butyl bromide	2.669	969877	128472	3537	1.5

4	Hyoscine butyl bromide	2.569	975488	128699	3683	1.5
5	Hyoscine butyl bromide	2.569	978547	128366	3599	1.5
6	Hyoscine butyl bromide	2.669	976899	128242	3537	1.5
Mean			975914			
Std. Dev			3286.898			
% RSD			0.336803			

Table: Results of Intermediate precision for Paracetamol:

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Paracetamol	3.845	5352142	563659	4686	1.4	1.7
2	Paracetamol	3.795	5365848	564588	4664	1.4	1.7
3	Paracetamol	3.855	5378413	563653	4653	1.4	1.7
4	Paracetamol	3.840	5378544	563548	4642	1.4	1.7
5	Paracetamol	3.855	5363599	565812	4668	1.4	1.7
6	Paracetamol	3.855	5386878	562542	4657	1.4	1.7
Mean			5370904				
Std. Dev			12656.44				
% RSD			0.235649				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

Accuracy:

Table: accuracy (recovery) data for Hyoscine butyl bromide

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	509439	15	15.042	100.274%	100.548%
100%	1010975.3	30	30.161	100.534%	
150%	1515818	45	45.378	100.843%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Table-: Accuracy (recovery) data for Paracetamol

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	347528	15	14.934	99.554%	99.936%
100%	609754	30	29.811	99.367%	
150%	884569	45	45.401	100.889%	

Acceptance Criteria:

- The % Recovery for each level should be between 98.0 to 102.0%.

Linearity

Chromatographic data for linearity study:

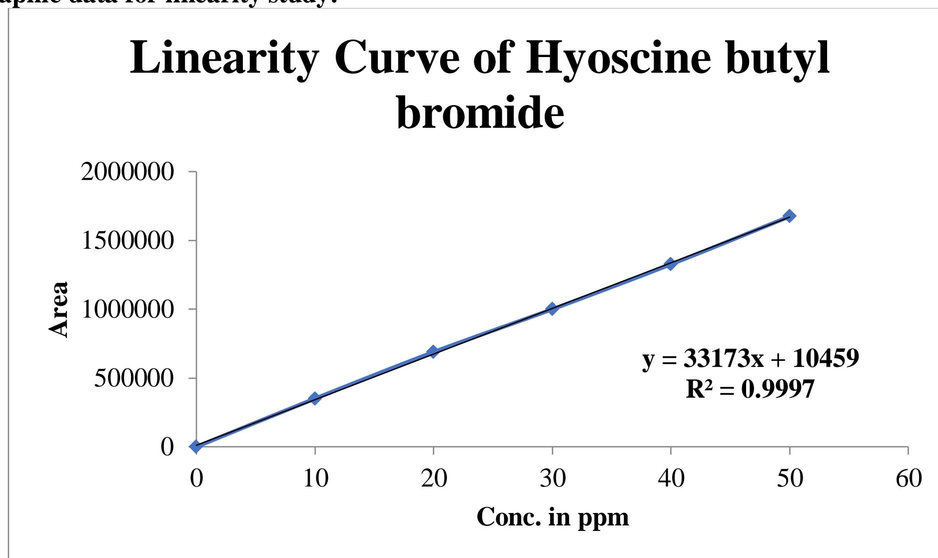

Figure: Calibration graph for Hyoscine butyl bromide

Linearity Results: (for Hyoscine butyl bromide)

Concentration(ppm)	Area
10	349878
20	688575
30	999896
40	1326523
50	1673878

Acceptance Criteria: Correlation coefficient should be not less than 0.998.

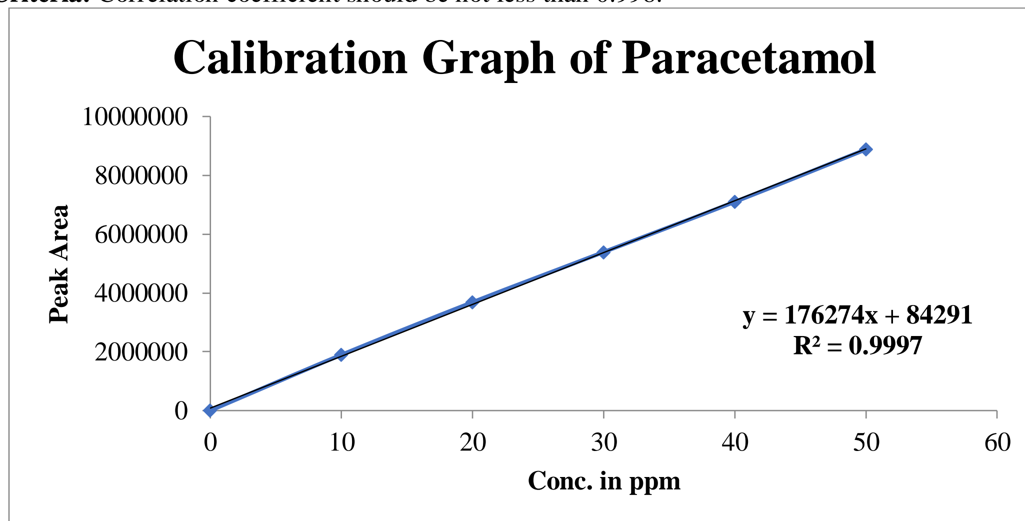

Figure: Calibration graph for Paracetamol

Linearity Results: (for Paracetamol)

Concentration (ppm)	Area
10	1896546
20	3685799
30	5389558
40	7096444
50	8878479

Acceptance Criteria:
Correlation coefficient should be not less than 0.99.

System suitability results for Hyoscine butyl bromide:

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4788.4	1.5
2	*Actual	3552.0	1.5
3	10% more	4636.6	1.5

System suitability results for Paracetamol:

S.No.	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	5866.8	1.4
2	*Actual	4674.7	1.4
3	10% more	5343.4	1.4

CONCLUSION:

A new reverse phase high performance liquid chromatographic (HPLC) method has been developed for the validation of Hyoscine butyl bromide and Paracetamol, both in their pure forms and in combined marketed formulations. The chromatographic analysis utilized a X bridge C18 column with dimensions of 4.6mm×150mm and 5µm particle size. The mobile phase consisted of a mixture of ACN (acetonitrile) and methanol (70:30% v/v), flowing at a rate of 1.2 ml/min, with detection at 260nm.

The method validation was conducted following ICH (International Council for Harmonisation) guidelines, covering parameters such as linearity, sensitivity, accuracy, precision, specificity, and robustness. The method demonstrated linear responses within concentration ranges of 20-60 mg/ml for Hyoscine butyl bromide and 10-30 mg/ml for Paracetamol. Both inter-day and intra-day precisions were found to be within acceptable limits. The precision of the method for assay determination was below 2.0% relative standard deviation (RSD).

In conclusion, the developed HPLC method offers a straightforward, rapid, and precise means of simultaneously quantifying Hyoscine butyl bromide and Paracetamol in pure form and in combined formulations. The use of a X bridge C18 column with a 70:30% v/v mixture of ACN: methanol as the mobile phase provided efficient separation and detection at 260nm. Compliance with ICH guidelines ensured thorough validation across various critical parameters including linearity, sensitivity, accuracy, precision, specificity, and robustness.

The method's demonstrated linear response over the specified concentration ranges, coupled with excellent precision (below 2.0% RSD), underscores its reliability for routine analysis in pharmaceutical quality control. This method is poised to contribute significantly to the assurance of product quality in the pharmaceutical industry, supporting both the

development and manufacturing stages of Hyoscine butyl bromide and Paracetamol formulations.

Acknowledgement:
The Authors are thankful to the Management and Principal, Department of Pharmacy, Samskruti college of pharmacy in Ghatkesar, Telangana, for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

BIBLIOGRAPHY:

1. Dr. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, (2002), PP 1-7.
2. A.BraithWait and F.J.Smith, Chromatographic Methods, 5th edition, Kluwer Academic Publisher, (1996), PP 1-2.
3. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition, Academic press, (1997), PP 24-37.
4. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, (2007), PP 15-23.
5. Chromatography, (online). URL:http://en.wikipedia.org/wiki/Chromatography.
6. Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, (2004), PP 7-8.
7. Sahajwalla CG a new drug development, vol 141, Marcel Dekker Inc., New York, (2004), PP 421–426.
8. D. H. Shewiy, E. Kaale, P. G. Risha, B. Dejaegher, J. S. Verbeke, Y. V. Heyden, Journal Pharmaceut. Biomed. Anal, 66, 2012, 11-23.
9. M. D. Rockville, General Tests, Chapter 621 – Chromatography System Suitability, United States Pharmacopeial Convention (USP), USP 31, 2009.
10. FDA Guidance for Industry-Analytical Procedures and Method Validation, Chemistry, Manufacturing, and Controls Documentation, Centre for Drug Evaluation and Research (CDER) and Centre for Biologics Evaluation and Research (CBER), 2000.
11. Korany MA, Mahgoub H, Ossama TF, Hadir MM. Application of artificial neural networks for response surface modelling in HPLC method development. J Adv Res, 3, 2012, 53-63.
12. Swartz ME, Jone MD, Fowler P, Andrew MA. Automated HPLC method development and transfer. Lc Gc N. Am, 75, 2002, 49-50.
13. Snyder LR, Kirkland JJ, Glajach JL. X. In Practical HPLC Methods Development. John Wiley, New York, 295, 1997, 643-712.
14. Swartz M, Murphy MB. New Fronties in Chromatography. Am Lab, 37, 2005, 22-27.
15. Dolan JW. Peak tailing and resolution. Lc Gc N. Am, 20, 2002, 430-436.