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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF DOXYLAMINE AND PYRIDOXINE IN PURE AND PHARMACEUTICAL DOSAGE FORM

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Abstract:

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Pyridoxine and Doxylamine, in its pure form as well as in tablet dosage form. Chromatography was carried out on an Sunfire C18 (4.6×250mm) 5µ column using a mixture of Water and Acetonitrile (60:40% v/v) as the mobile phase at a flow rate of 0.9ml/min, the detection was carried out at 220nm. The retention time of the Doxylamine and Pyridoxine was 3.0, 3.8±0.02min respectively. The method produce linear responses in the concentration range of 5-25µg/ml of Doxylamine and 5-25µg/ml of Pyridoxine. 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: *Doxylamine, Pyridoxine, RP-HPLC, validation.*

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INTRODUCTION:

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components. [1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance. [2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable. [1, 2]

DIFFERENT METHODS OF ANALYSIS:

The following techniques are available for separation and analysis of components of interest.

Spectral methods:

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted by the sample.

E.g. UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry.2

Electro analytical methods:

Electro analytical methods involved in the measurement of current voltage or resistanceas a property of concentration of the component in solution mixture.

E.g. Potentiometry, Conductometry, Amperometry.

Chromatographic methods [15]
Chromatography is a technique in which chemicals in solutions travel down columns or over surface by means of liquids or gases and are separated from each other due to their molecular characteristics.

E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC).

MATERIALS AND METHOD:

Doxylamine from Sura labs, Pyridoxine from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck,

Hplc method development:

Trails

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Doxylamine and Pyridoxine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.15ml of the Doxylamine and 0.15ml of the Pyridoxine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water and Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Water in proportion 40:60 v/v respectively.

Optimization of Column:

The method was performed with various columns like Symmetry, Hypersil and Sunfire C18 (4.6×150mm, 5µ) 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.
Temperature : 35ºC
Column : Sunfire C18 (4.6×250mm) 5µ
Mobile phase : Acetonitrile: Water (40:60v/v)
Flow rate : 0.9ml/min
Wavelength : 220nm
Injection volume : 10 µl
Run time : 6min

Validation

Preparation of mobile phase:

Preparation of mobile phase:

Accurately measured 600ml (60%) of Water, 400ml of Acetonitrile (40%) 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:

Optimized Chromatogram (Standard)

Mobile phase ratio : Acetonitrile: Water (40:60v/v)
Column : Sunfire C18 (4.6×250mm) 5µ
Column temperature : 35ºC
Wavelength : 220nm
Flow rate : 0.9ml/min
Injection volume : 10µl
Run time : 6minutes

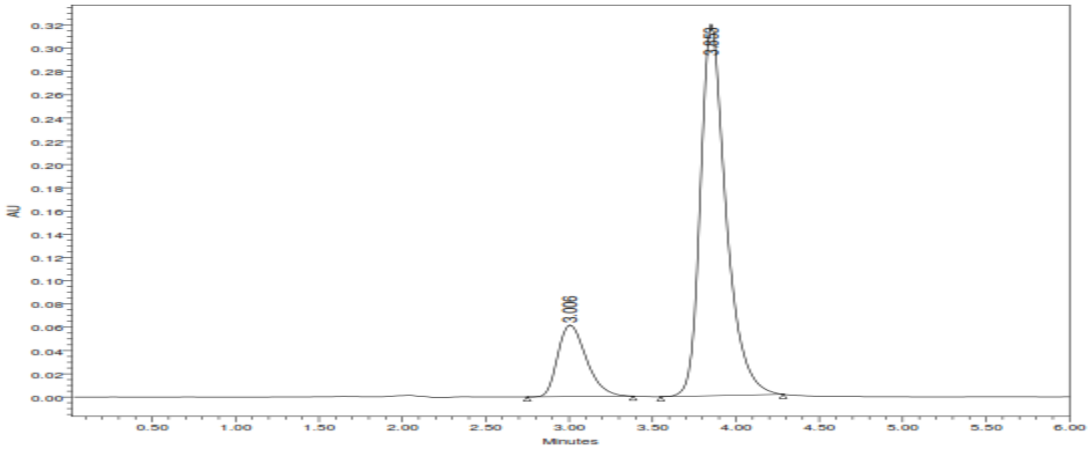

Fig: Optimized Chromatogram (Standard)

Table: Optimized Chromatogram (Standard)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Doxylamine	3.006	731322	61677	1.2	8574
2	Pyridoxine	3.853	3421257	319786	1.1	9664

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Optimized Chromatogram (Sample)

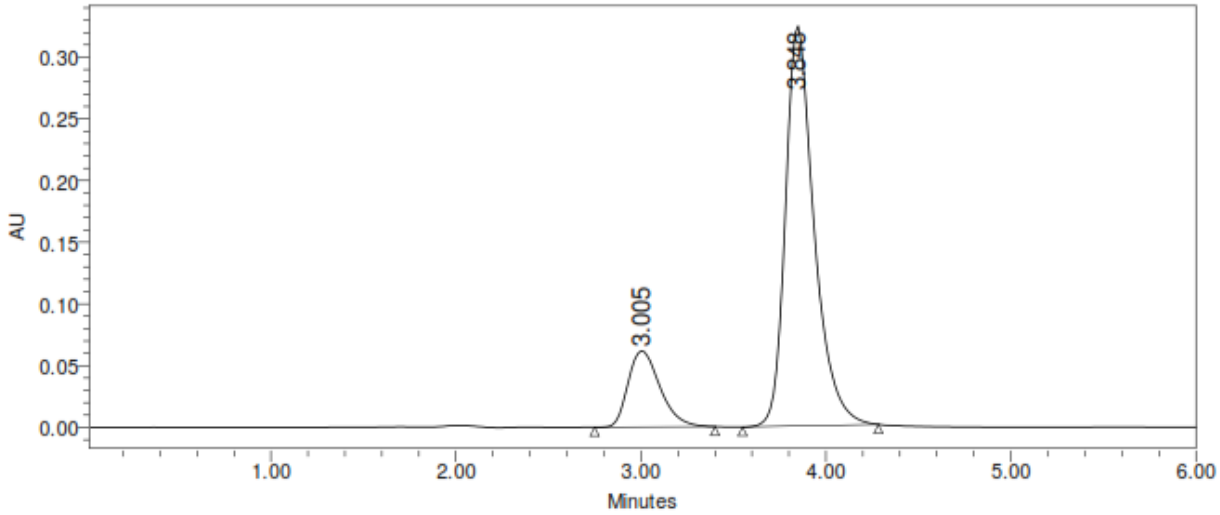

Fig: Optimized Chromatogram (Sample)

Table : Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Doxylamine	3.005	658995	61772	1.1	7442
2	Pyridoxine	3.848	3096188	324054	1.2	7331

Acceptance criteria:

- Theoretical plates must be not less than 2000
- Tailing factor must be not less than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

Assay (Standard):

Table : Results of system suitability Doxylamine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Doxylamine	3.008	658263	61335	7462	1.2
2	Doxylamine	3.009	658264	61947	8264	1.1
3	Doxylamine	3.008	653426	61049	6627	1.2
4	Doxylamine	3.010	653058	61141	7264	1.1
5	Doxylamine	3.006	657393	61735	6645	1.1
Mean			656080.8			
Std. Dev.			2618.946			
% RSD			0.39918			

Acceptance criteria:

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

Table: Results of system suitability Pyridoxine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Pyridoxine	3.857	3028176	381011	9583	1.1
2	Pyridoxine	3.859	3018373	381645	8927	1.2
3	Pyridoxine	3.857	3018462	381663	8465	1.1
4	Pyridoxine	3.861	3081711	381746	9222	1.2
5	Pyridoxine	3.853	3075143	381193	8462	1.1
Mean			3044373			
Std. Dev.			31427.07			
% RSD			1.0323			

Acceptance criteria:

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

Assay (Sample):

Table : Peak results for Assay sample of Doxylamine

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Doxylamine	3.008	651712	61173	1.2	8563
2	Doxylamine	3.005	657635	61936	1.1	7462
3	Doxylamine	3.007	658917	61196	1.1	9264

Table : Peak results for Assay sample of Pyridoxine

S.No	Name	RT	Area	Height	USP Tailing	USP Plate
1	Pyridoxine	3.854	3029472	361938	1.1	6476
2	Pyridoxine	3.853	3017462	361746	1.1	7264
3	Pyridoxine	3.855	3028171	371864	1.2	6545

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

=429729/423559.5*10/15*15/0.0986*99.2/100*0.3944/40*100

The % purity of Doxylamine and Pyridoxine in pharmaceutical dosage form was found to be 100.5%

LINEARITY

Table : CHROMATOGRAPHIC DATA FOR LINEARITY STUDY FOR DOXYLAMINE:

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	5	230247
66.6	10	462332
100	15	659905
133.3	20	892989
166.6	25	1101075

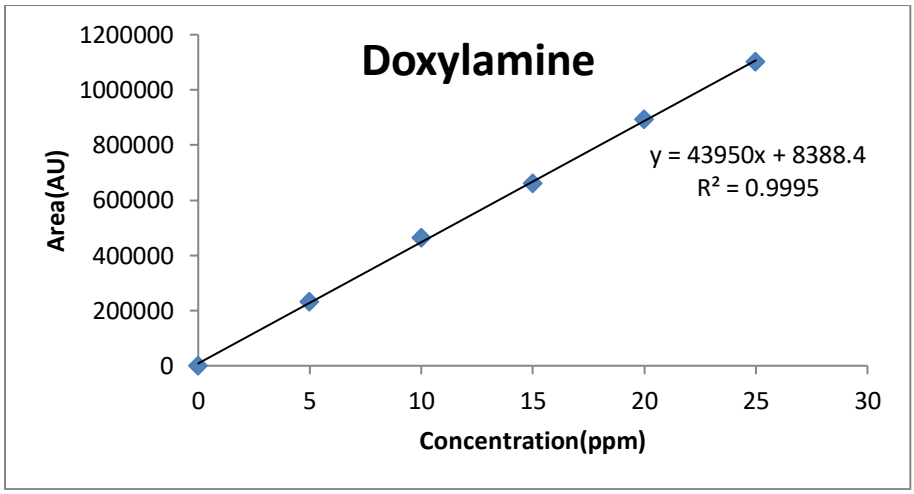

Fig: Chromatogram showing linearity level

Table : Chromatographic Data for Linearity Study PYRIDOXINE:

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	5	1215225
66.6	10	2135937
100	15	3020839
133.3	20	4078841
166.6	25	5058145

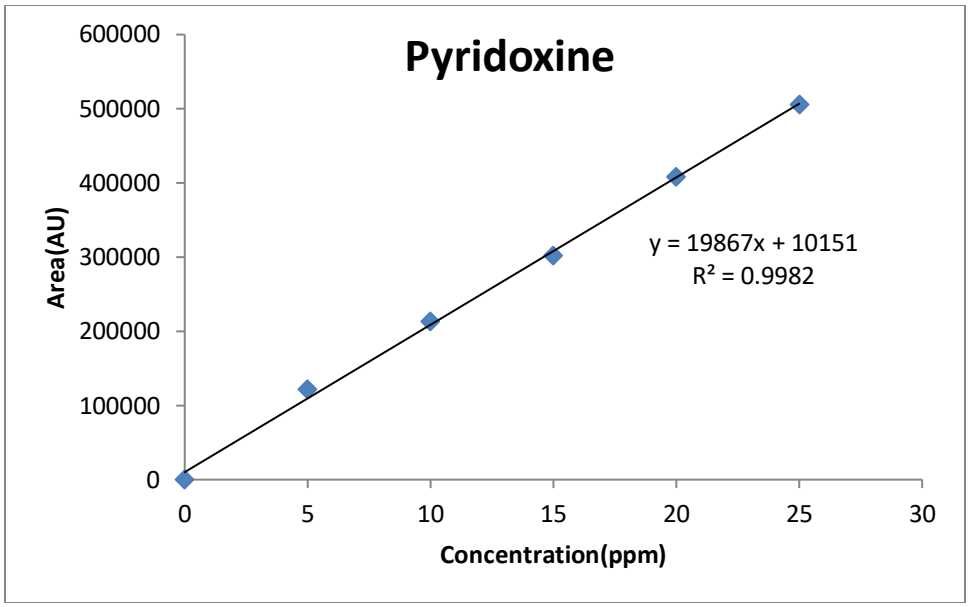

Fig: Chromatogram showing linearity level

REPEATABILITY

Table : Results of repeatability for Doxylamine:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Doxylamine	3.003	654426	61521	8474	1.1
2	Doxylamine	3.005	659862	61937	8262	1.2
3	Doxylamine	3.007	650837	62018	8117	1.1
4	Doxylamine	3.008	651433	61893	7917	1.2
5	Doxylamine	3.005	652752	61867	8011	1.1
Mean			653862			
Std.dev			3626.323			
%RSD			0.554601			

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.

Table: Results of repeatability for Pyridoxine:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Pyridoxine	3.851	3028371	381736	6881	1.1
2	Pyridoxine	3.852	3009188	380138	9363	1.2
3	Pyridoxine	3.854	3067464	386615	7844	1.1
4	Pyridoxine	3.853	3076611	380183	9746	1.2
5	Pyridoxine	3.851	3011912	379471	7883	1.2
Mean			3038709			
Std.dev			31463.69			
%RSD			1.035429			

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:

Table : Results of Intermediate precision day1 for Doxylamine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Doxylamine	3.007	658911	60173	9141	1.1
2	Doxylamine	3.005	650383	61936	9662	1.2
3	Doxylamine	3.005	658813	60383	9746	1.1
4	Doxylamine	3.005	651138	60774	7746	1.1
5	Doxylamine	3.005	659937	61947	8264	1.2
6	Doxylamine	3.010	653715	61893	7836	1.1
Mean			655482.8			
Std. Dev.			4258.945			
% RSD			0.649742			

Acceptance criteria:

%RSD of Six different sample solutions should not more than 2

Table : Results of Intermediate precision day1 for Pyridoxine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Pyridoxine	3.851	3021731	369771	8564	1.1
2	Pyridoxine	3.848	3019183	372746	9227	1.1
3	Pyridoxine	3.848	3029847	371866	7565	1.2
4	Pyridoxine	3.850	3028471	369017	7726	1.1
5	Pyridoxine	3.849	3088641	376453	6746	1.2
6	Pyridoxine	3.860	3056633	386621	5977	1.1
Mean			3040751			
Std. Dev.			26990.09			
% RSD			0.887613			

Acceptance criteria:

- %RSD of Six different sample solutions should not more than 2.

Table : Results of Intermediate precision Day 2 for Doxylamine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Doxylamine	3.006	648822	61847	6983	1.1
2	Doxylamine	3.008	640863	59882	7728	1.2
3	Doxylamine	3.008	643382	60774	9576	1.1
4	Doxylamine	3.007	641884	58928	8275	1.2
5	Doxylamine	3.007	647822	61483	9837	1.1
6	Doxylamine	3.005	649181	60928	8744	1.2
Mean			645325.7			
Std. Dev.			3711.009			
% RSD			0.57506			

Acceptance criteria:
%RSD of Six different sample solutions should not more than 2 Table:

Table: Results of Intermediate precision Day 2 for Pyridoxine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Pyridoxine	3.853	3075833	389911	7039	1.1
2	Pyridoxine	3.857	3029583	379019	9857	1.2
3	Pyridoxine	3.854	3021991	381875	7881	1.1
4	Pyridoxine	3.855	3022485	391099	7902	1.2
5	Pyridoxine	3.854	3085833	389222	9285	1.1
6	Pyridoxine	3.853	3019482	391184	8955	1.2
Mean			3042535			
Std. Dev.			30022.42			
% RSD			0.986757			

Acceptance criteria:
- %RSD of Six different sample solutions should not more than 2

ACCURACY:

Table :The accuracy results for Doxylamine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	331938	7.5	7.3	99.88	100.166
100%	658274	15	14.7	98.89	
150%	970963	22.5	22.2	101	

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: The accuracy results for Pyridoxine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	209357	7.5	7.49	99.7%	99%
100%	420697.7	15	14.9	99%	
150%	631550.7	22.5	22.48	99%	

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.

Robustness

Table : Results for Robustness -Doxylamine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	658211	3.006	8793	1.2
Less Flow rate of 0.8mL/min	621077	3.441	7269	1.3
More Flow rate of 1.0mL/min More Flow rate of 0.9mL/min	642190	2.663	9446	1.2
Less organic phase	542402	3.185	8126	1.1
More organic phase	642112	2.867	5854	1.3

Table: Results for Robustness-Pyridoxine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	429069	3.853	5224	1.59
Less Flow rate of 0.8mL/min	472673	4.426	6328	1.58
More Flow rate of 1.0mL/min	392497	3.415	6217	1.54
Less organic phase	391379	4.291	6996	1.61
More organic phase	391703	3.583	6120	1.50

Acceptance criteria:
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION:

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Doxylamine and Pyridoxine in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Doxylamine and Pyridoxine was freely soluble in ethanol, methanol and sparingly soluble in water.

Water and Acetonitrile (60:40% v/v) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of Doxylamine and Pyridoxine in bulk drug and in Pharmaceutical dosage forms.

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