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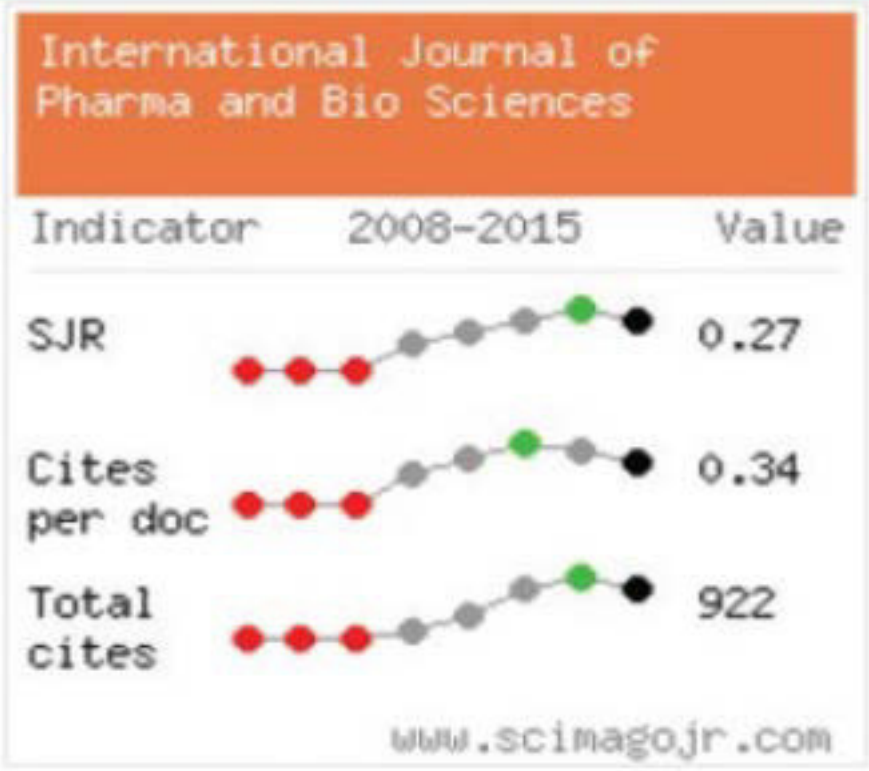

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DEVELOPMENT OF A SIMPLE, RAPID AND SPECIFIC RP-HPLC METHOD FOR THE ESTIMATION OF PYRIDOXINE HYDROCHLORIDE AND DOXYLAMINE SUCCINATE IN BULK AND COMBINED PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A rapid and specific method was developed and validated for the quantitation of Pyridoxine Hydrochloride and Doxylamine in bulk and combined pharmaceutical dosage forms. To acheive the chromatographic separation Shimadzu system has been used, equipped with LC-20AD high-performance liquid chromatography, degasser PGU-20A 5, variable wavelength programmable diode array detector SPD-M20A, auto sampler SIL-20 AC HT, and column oven CTO-10 A5 VP hic separation was achieved by using Shimzadu prominence LC-20AD high-performance liquid chromatography, equipped with degasser PGU-20A 5, variable wavelength programmable diode array detector SPD-M20A, auto sampler SIL-20 AC HT, and column oven CTO-10 A5 VP. XBridge C18, 4.6 mm X150 mm, 5 microns Make: Waters, Water's Corporation. Inc. The proposed method was validated for selectivity, precision, linearity and accuracy. The developed method was successfully applied to estimate the amount of Pyridoxine Hydrochloride and Doxylamine in bulk and combined dosage forms.

KEYWORDS: ***RP-HPLC, Pyridoxine Hydrochloride (PYH), Doxylamine Succinate (DXA).***

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INTRODUCTION

Pyridoxine hydrochloride (PYH)[1] s chemically 3, 4-pyridinediacetonitrile, 5-hydroxy-6-methyl, hydrochloride (Figure 1). It is a water-soluble vitamin and involved principally in amino acid, carbohydrate, and fat metabolism[2]. It is also required for the formation of haemoglobin.[3,4,5,] Doxylamine is a first-generation antihistamine.[4,5] It can be used by itself as a short-term sedative and in combination with other drugs to provide night-time allergy and cold relief. Doxylamine is also used in combination with the analgesics paracetamol (acetaminophen) and codeine as an analgesic/calmative preparation, and is prescribed in combination with vitamin B6 (pyridoxine) to prevent morning sickness in pregnant women. HPLC methods are useful in the determination of drugs in pharmaceutical formulations especially with those containing more than one active components. Therefore, the aim of this work was to develop a relatively simple HPLC method for simultaneous quantification of Pyridoxine hydrochloride (PYH) and Doxylamine (DXA). This paper describes the development and validation of reliable, simple, stable and economic reverse phase HPLC assay suitable for quality control in pharmaceutical industry due to its sensitivity, simplicity, selectivity and lack of excipients interference.

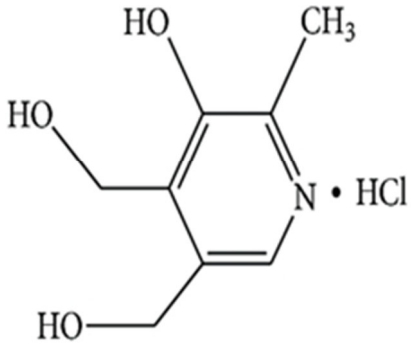


Figure 1
Chemical structure of Pyridoxine HCL

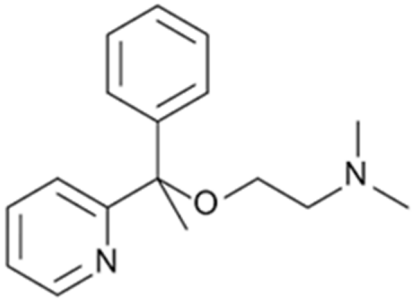


Figure 2
Chemical structure of Doxylamine

MATERIALS AND METHODS

Pure standards of PYH and DXA were obtained from Sigma-Aldrich Co.

Instrument and Chromatographic Condition

To acheive the chromatographic separation Shimadzu system has been used, equipped with LC-20AD high-performance liquid chromatography, degasser PGU-20A 5, variable wavelength programmable diode array detector SPD-M20A, auto sampler SIL-20 AC HT, and column oven CTO-10 A5 VP. XBridge C18, 4.6 mm X150 mm, 5 microns Chromatographic separation was achieved by using Shimzadu prominence LC-20AD high-performance liquid chromatography, equipped with degasser PGU-20A 5, variable wavelength programmable diode array detector SPD-M20A, auto sampler SIL-20 AC HT, and column oven CTO-10 A5 VP. XBridge C18, 4.6X150 mm, Make: Water's Corporation. Inc The column temperature was kept at 30^0 C^o, and the mobile phase flow rate was maintained at 1.0 mL/min. The detector was Setat 210 nm.[6]The injection volume was 10 µL, and the run time was 20 min for each injection. Other instruments such as pH meter, electronic weighing balance, and ultrasonic bath were also used.

Diluent Preparation

Diluent – 1
250 mg of sodium hydroxide in 1000 mL water

Diluent – 2
2 mL of orthophosphoric acid in 1000 mL of water

Standard stock preparation

PYH and DXA were weighed (25 mg each) and transferred to two separate 50 ml volumetric flasks and about 5 mL of diluent-1 and 30 ml of diluent -2 was added and sonicated to dissolve. Volumes were made up to the mark with diluent-2 Aliquot from the stock solution of PYH was appropriately diluted with mobile phase to obtain working standard of 100 µg mL^{-1} of PYH and same way for DXA.

Assay sample preparation

Weigh accurately and transfer 10 numbers of Doxylamine Succinate and Pyridoxine Hydrochloride delayed release tablets into a 200 mL volumetric flask. Add 20 mL of diluent-1 and sonicate with intermittent shaking until to disintegrate. Add 120 mL of diluent-2 and sonicate for 15 minutes with intermittent shaking. Shake mechanically for 15 minutes. Dilute to volume with diluent-2 and mix well. Centrifuge a portion of the above solution at 3000 rpm for 10 minutes. Dilute 5 mL of the above supernatant solution to 25 mL with diluent-2 and mix well. Filter through 0.45 µm PVDF filter by discarding the first 4 mL of the filtrate. (Concentration of about 100 µg/mL of Doxylamine Succinate and Pyridoxine Hydrochloride).[6,7]

Optimization of chromatographic condition

Mode of operation: Gradient
Column: XBridge C18, 4.6 mm X 150 mm, 5.0 µm.
Make: Water's Corporation.Inc ,
Flow rate: 1.0 ml/min
Column temperature: 30^0 C
Sample port temperature: 25^0C
Injection volume: 10µL
Run time: 20 minutes
Retention times: 4.6 minutes and 11.5 minutes for PYH and DXA respectively.

Gradient programme

Composition of solvent for gradient programme

Time (min)	% of Mobile Phase A	% of Mobile Phase B
0	100	0
12	40	60
14	100	0
20	100	0

Method validation

The method was validated in terms of the following parameters, system suitability, linearity, LOD, LOQ, specificity, accuracy, precision, robustness and ruggedness as per the ICH guidelines.

System Suitability

The chromatographic conditions were set as per the optimized parameters and steady baseline. Six replicates of working standard solution are injected and the Chromatograms are the mobile phase was allowed to equilibrate with stationary phase as was indicated by the recorded. The % relative standard deviation (%RSD).[8] of retention time, asymmetry, theoretical plate count and peak areas were determined and the results were shown in Table 1.

Linearity

Accurately measured volume of the standard stock solution was diluted with diluents to get the final concentrations of Standard PYH as 5-25µg/ml and Doxylamine succinate standard as 20-100µg/ml respectively. Six different concentrations of the mixed standard drugs of Pyridoxine HCL and DXA were prepared for linearity studies and injected into the system (n=6). The response was measured as peak areas. Each concentration was prepared from individual stock solution. The peak areas were plotted versus concentrations to get the calibration curve.

Detection limit and quantification limit [LOD and LOQ]

The sensitivity of the simultaneous method of Pyridoxine hCL and Doxylamine succinate is estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). The LOD and LOQ[9] were calculated using the formula.

LOD = 3.3 x σ/S
LOQ = 10 x σ/S
σ = Standard Deviation of Response
S = Slope of the calibration curve
The results were shown in Table.1 standard

Specificity

The specificity of the method was performed by injecting a blank solution (without any sample) and then a drug solution of 10µl injected into the column, under optimized chromatographic conditions, to demonstrate the separation of both Pyridoxine hcl and Doxylamine.

Accuracy

The accuracy was carried out by adding known amounts of each analyte corresponding to three concentration levels (80%, 100%, 120%)[11-13] of the label claim to the excipients. At each level, six determinations were determined and the results are expressed as a percentage. Analyte recovered by the proposed method. The results are given in the Table.3.

Precision

The precision of the method was assessed by studying intra-day and inter-day variation. In the intra-day studies, standard and sample solutions were analyzed in triplicate on the same day and % RSD was calculated. In case of inter-day studies, standard and sample solutions were analyzed in triplicate on three consecutive days and % RSD were calculated. The results are shown in the table.4

Robustness

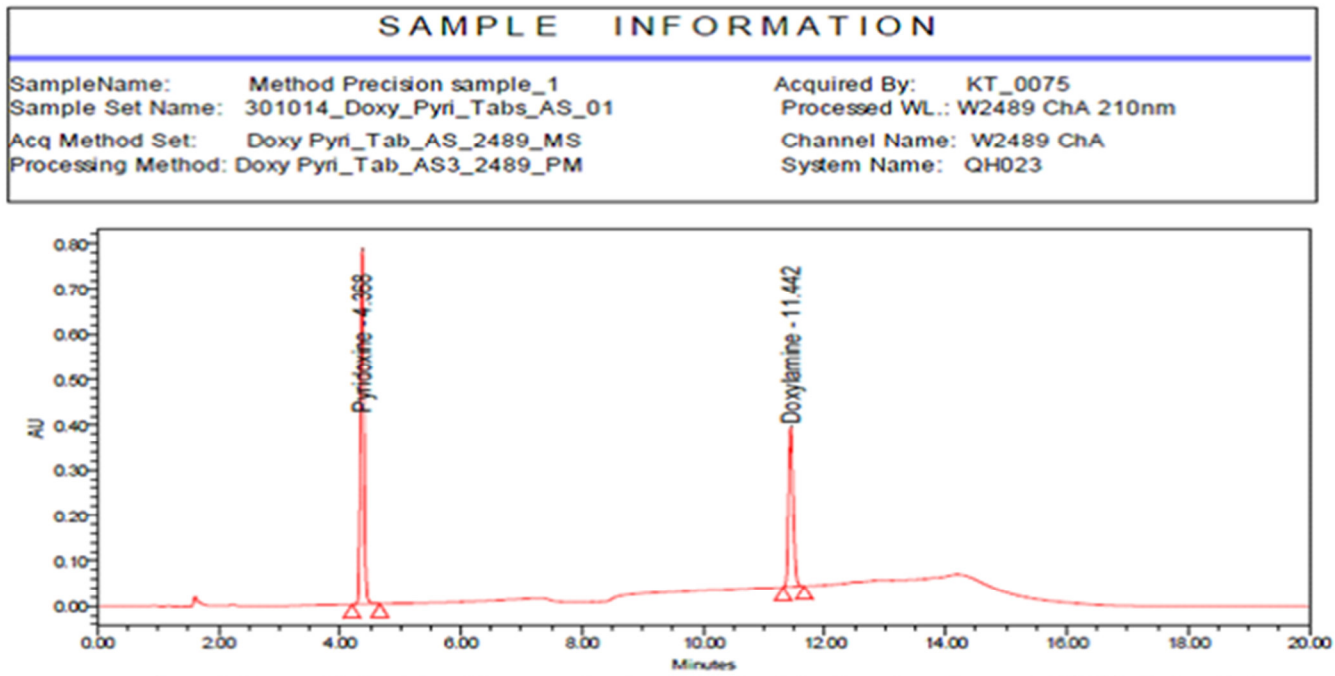
Robustness is a measure of capacity of analytical methods to remain unaffected by small deliberate variation of the operating conditions. It was tested by changing the flow rate, temperature and wavelength by ±2 nm. The results are shown in the Table .5

Ruggedness

The ruggedness of the method was analyzed in different days and different chemists to check for any changes in the chromatograph, % RSD for the retention time and the area was calculated. The results are shown in the Table.6.

RESULTS AND DISCUSSIONS

Method Validation



	Name	Vial	Injection	RT	Area (µV*sec)	% Area	USP Resolution	Int Type
1	Pyridoxine	4	1	4.368	3111660	62.72		BB
2	Doxylamine	4	1	11.442	1849793	37.28	57.41	BB

Figure 3
Optimised chromatogram of Pyridoxine Hcl and andDoxylamine.

System Suitability

The relative standard deviation (RSD) of Doxylamine and Pyridoxine peak area is NMT 2.0% from standard preparation. The USP plate and Pyridoxine peak is NLT counts for Doxylamine 5000 from standard preparation. The tailing factor for Doxylamine and Pyridoxine peak is NMT 2.0 from standard preparation.

Table 1
System suitability.

Injection No	Peak area for Doxylamine	Peak area for Pyridoxine
1	1896503	3185563
2	1893412	3160400
3	1854186	3106135
4	1869798	3142209
5	1864999	3138109
Mean	1875779	3146483
% RSD	0.98	0.93
USP Tailing factor	1.18	1.06
USP Plate count	107964	28327

Linearity Determination

Solutions of DXA and PYH at concentration levels from about 50% to 200% of standard solution were injected into HPLC system. The linearity graph was plotted from 50% to 200%. Six injections were performed at 50% level and at 200% level.[14-16]

RESULTS

Linearity and range of assay method is established by injecting series solutions of Doxylamine Succinate and Pyridoxine Hydrochloride. The data is shown in Table 2 and 3.

Table 2
Linearity study for Doxylamine Succinate.

Sample No.	% level	Concentration (µg/mL)	Mean Peak Area
1	50	51.344	965375
2	75	77.017	1437913
3	80	82.151	1527854
4	100	102.689	1938429
5	120	123.227	2298332
6	150	154.034	2938521
7	200	205.379	3854584

Table 3
Linearity study for Pyridoxine HCL.

Sample No.	% level	Concentration (µg/mL)	Mean Peak Area
1	50	51.192	1632788
2	75	76.788	2431554
3	80	81.907	2585474
4	100	102.384	3274215
5	120	122.861	3868837
6	150	153.577	4928177
7	200	204.769	6419923

Table 4
Linearity Plot of Doxylamine Succinate.

Linear Regression Analysis for Doxylamine	Concentration in µg/mL vs. Area
Correlation Coefficient Square (r^2)	0.9995
Slope	18905.870
Y-Intercept	12151.215

Table 5
Linearity Plot of Pyridoxine Hydrochloride.

Linear Regression Analysis for Pyridoxine	Concentration in µg/mL vs. Area
Correlation Coefficient Square (r^2)	0.9994
Slope	31403.857
Y-Intercept	31813.946

Detection limit and quantification limit (LOD and LOQ)

Pyridoxine Hydrochloride

LOQ: 0.143 µg/mL
LOD: 0.047 µg/mL

Doxylamine Succinate

LOQ: 0.255 µg/mL
LOD: 0.084 µg/mL

Specificity

The specificity of the RP-HPLC method was determined by the complete separation of Pyridoxine HCL ((PYH) and Doxylamine succinate (DXA)[17,18]as shown in Figure 5. The peaks obtained for (PYH)and DXA were sharp and have a clear baseline separation.

Accuracy

Known amount of DXA and PYH were spiked with placebo for Doxylamine Succinate and Pyridoxine Hydrochloride Delayed Release Tablets, 10mg/ml,in order to produce recovery at 50%, 100 % and 200% levels of the DXA and PYH working concentration 100µg/mL. Spiked assay samples were prepared in triplicate, injected in duplicate and the percentage recovery was calculated.

Table 6
Method accuracy study- Doxylamine Succinate.

Sample No.	Theoretical (%)	Mean Peak Area	% Recovery	Mean (%) Recovery	% RSD
1	50	893103	98.42	97.99	
2	50	883742	97.54		0.44
3	50	888112	98.01		
1	100	1764581	97.80	97.76	
2	100	1770794	98.07		0.32
3	100	1759898	97.43		
1	200	3537017	97.27	98.28	
2	200	3562222	98.71		0.89
3	200	3566293	98.86		

Table 7
Method accuracy study- Pyridoxine Hydrochloride.

Sample No.	Theoretical (%)	Mean Peak area	% Recovery	Mean (%) Recovery	% RSD
1	50	1515131	102.12		
2	50	1508084	101.57	101.47	0.69
3	50	1515042	100.72		
1	100	3014239	102.19		
2	100	3025362	102.54	102.08	0.51
3	100	3001739	101.51		
1	200	5891677	99.81		
2	200	5931930	100.55	100.30	0.42
3	200	5930250	100.56		

Precision

Precision of the assay method was determined by injecting, in duplicate, six individual sample solutions of Doxylamine Succinate and Pyridoxine Hydrochloride Delayed Release Tablets, 10mg/ml.The samples were prepared as per the method.

Table 8
Precision study Doxylamine Succinate and Pyridoxine Hydrochloride.

Sample No	DoxylamineSuccinate		Pyridoxine Hydrochloride	
	Mean peak area	% assay	Mean peak area	% assay
1	1849235	99.02	3111204	100.04
2	1842286	98.65	3103431	99.79
3	1824276	97.68	3103350	99.79
4	1906478	102.08	3165773	101.80
5	1899109	101.69	3200017	102.90
6	1870864	100.18	3176516	102.14
Mean	NA	99.88	NA	101.07
%RSD	NA	1.75	NA	1.35

Robustness

Standard solution was prepared and injected into the chromatographic system as per the conditions specified in the method. The same standard solution was re-injected by changing one parameter at a time, keeping other parameters constant. A set of system suitability data was calculated for standards injected under altered method conditions and compared against the values generated under normal method conditions.

Method Parameters

Flow Rate (Normal flow is 1.0 mL/min)
Flow minus → 0.9 mL/min
Flow plus → 1.1 mL/min
Column Operating Temperature (Normal temperature is 30°C)
Temperature minus → 25° C
Temperature plus → 35° C

Table 9
Robustness study- Retention time of Doxylamine Succinate.

Parameters		Retention Time (min)	Mean Peak area (n=5)	%RSD	USP Tailing factor	USP Plate count
Normal Condition	1.0 mL/min, 30°C	11.367	1947150	0.13	1.19	108113
Flow Rate Minus	0.9 mL/min	11.816	2139573	0.11	1.20	112874
Flow Rate Plus	1.1 mL/min	11.216	1948289	0.06	1.19	107352
Column Temperature Minus	25°C	11.520	1945738	0.13	1.19	108851
Column Temperature Plus	35°C	11.220	1945016	0.14	1.04	110073

Table 10
Robustness study - Retention time of Pyridoxine Hydrochloride.

Parameters		Retention Time (min)	Mean Peak area (n=5)	%RSD	USP Tailing factor	USP Plate count
Normal Condition	1.0 mL/min, 30°C	4.289	3376835	0.08	1.05	23438
Flow Rate Minus	0.9 mL/min	4.650	3737177	0.06	1.07	26331
Flow Rate Plus	1.1 mL/min	4.168	3378083	0.08	1.04	22504
Column Temperature Minus	25°C	4.398	3386147	0.09	1.05	24330
Column Temperature Plus	35°C	4.171	3371221	0.13	1.20	22448

CONCLUSION

The proposed method was validated in accordance with ICH guidelines and all the results obtained with this method are all within the limits. This method can be suitable for routine analysis in laboratories. Therefore, this method is simple, accurate, rapid, reliable method for simultaneous estimation of Pyridoxine HCL and Doxylamine Succinate.

AUTHOR CONTRIBUTION STATEMENT

Conceived and designed the experiments: Amar Kumar Kasturi and N.Balakrishnan. Performed the experiments: Amar Kumar Kasturi. Analyzed the data: Amar Kumar Kasturi, Kanaka raju Medicherla and N. Balakrishnan. Contributed reagents/materials/analysis tools: N. Balakrishnan. Wrote the paper: Amar Kumar Kasturi and Kanaka raju Medicherla.

CONFLICT OF INTEREST

Conflict of interest declared none.

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