



Development and Validation of RP-HPLC Method for Simultaneous Estimation of Doxylamine Succinate and Pyridoxine Hydrochloride in Bulk and Pharmaceutical Dosage Forms

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ABSTRACT

The present paper illustrates about development and validation of a new, simple, precise and accurate RP-HPLC method with enhanced sensitivity for the simultaneous determination of Doxylamine Succinate (DAS) and Pyridoxine Hydrochloride (PDH) in bulk and its dosage forms. The drug showed good absorbance in mobile phase at 263 nm. Under the optimized conditions, linear relationship with good correlation coefficient (0.9994 and 0.9992 for DAS and PDH respectively) was found between the concentration range of 10-50 µg/ml for DAS and 5-25 µg/ml for PDH. The limit of detection for the method was 1.8 and 0.4 µg/ml for DAS and PDH respectively. The limit of quantification for the method was observed to be 4.4 and 4.2 µg/ml for DAS and PDH respectively. The precision of the method was satisfactory; the values of relative standard deviations did not exceed 2%. The recovery values were 99.4-99.8% ± 1.61% for DAS and 99.8-100.4% ± 0.12% for PDH. The chromatographic method was developed on AGILENT HPLC with UV detection. The method was optimized by Kromosil-C₁₈, (250 * 4.6mm, 5µ) column by using phosphate buffer (pH-5); methanol (40:60) as a mobile phase with 1 ml/min as flow rate. The detection wavelength is 263 nm. The proposed method are successfully applied for the determination of DAS and PDH in bulk and their dosage forms. The method is having higher sensitivity and wider linear range. The proposed method is practical and valuable for its routine application in quality control laboratories for estimation of DAS and PDH.

Keywords: RP-HPLC, validation, precision, accuracy

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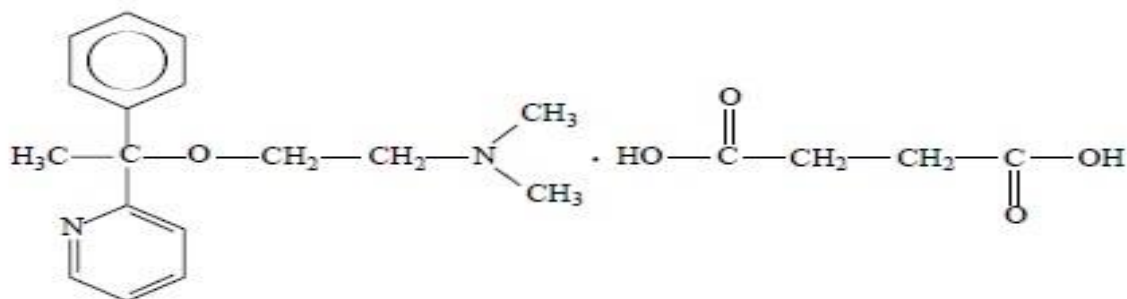


Figure 1. Chemical structure of Doxylamine Succinate

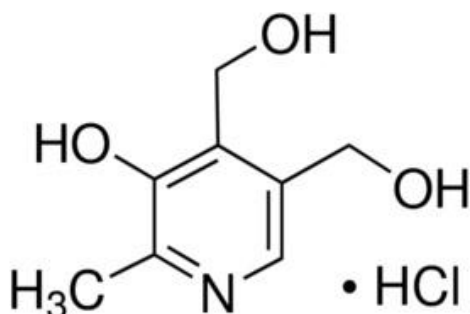


Figure 2. Chemical structure of Pyridoxine Hydrochloride

INTRODUCTION

Doxylamine Succinate is N,N- Dimethyl-2-[(α -methyl- α -2 pyridinyl) benzyloxy] ethylamine hydrogen Succinate (**Figure 1**) which is an antihistamine and Pyridoxine Hydrochloride is chemically known as 4,5-Bis[(hydroxyl methyl)-2-methyl pyridine-3-ol] Hydrochloride salt (**Figure 2**) assists in the balancing of sodium and potassium as well as promoting red blood cells production [1]. However, many methods for the estimation of these drugs like UV [1-4], RP-HPLC [5-7], stability indicating HPLC [8] was reported in the literature. But the present method is more sensitive and precise than the reported methods.

MATERIALS AND METHODS

Equipment used

A HPLC (Agilent technology) consisting of uv detector, symmetry Kromosil C₁₈ 250mm×4.6mm, 5 μ m packing of octadecylsilane chemically bonded to porous silica particles and ezochrome 2 software. Axis Ag N 204-PO Digital Balance was used to weigh samples with accuracy. 1.5 LH Ultra Sonic Bath sonicator was used to degas the mobile phase. Membrane filters of pore size 0.45 μ m were used to filter the samples solution. Methanol (HPLC grade) was supplied by Merck specialities Pvt., Ltd. Water (HPLC grade) was supplied by Merck specialities Pvt., Ltd. Potassium Dihydrogen Phosphate Buffer, Standard drugs of DAS and

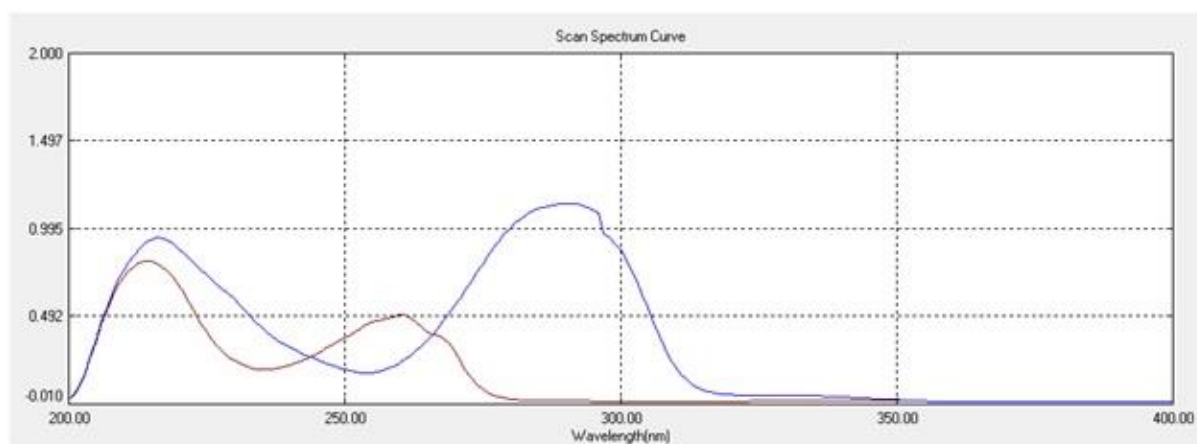


Figure 3. Overlain Spectra of Pyridoxine Hydrochloride and Doxylamine Succinate

PDH (gift samples) were supplied by Mylan laboratories Pvt. Ltd., Hyderabad. Tablets containing 10 mg doxylamine succinate and 10 mg pyridoxine hydrochloride (Diclegis) were procured from local market.

Method development

The pure drugs of DAS and PDH were injected in to the isocratic HPLC system and run at different solvent systems. Different mobile phases like methanol and water, Acetonitrile and water, methanol and Acetonitrile were tried in order to find the best conditions for the elution. It was found that Potassium Dihydrogen phosphate buffer (pH-5) and Methanol gave satisfactory results as compared to other mobile phases. The mobile phase system was tried with different proportions and using different flow rates. Finally, the optimal composition of the mobile phase was determined to be 20 mM potassium di hydrogen phosphate buffer (pH-5) and Methanol in the ratio of 40:60 v/v at a flow rate of 1.0 ml/min on Kromosil C18 (250 x 4.6 mm id; 5 μ). The detection wavelength was selected as 294 nm based on UV spectrum. The run time was set as 10 minutes.

Preparation of stock Solution

The separate stock solutions of DAS and PDH were prepared by accurately weighing 25mg each into a separate 25 ml volumetric flasks A and B and made up to the volume with mobile phase to get 1000 μ g/ml respectively.

Selection of Analytical Wavelength

By appropriate dilution of each standard stock solution with mobile phase, various concentrations of DAS and PDH were prepared separately. Each solution was scanned using double beam UV visible spectrophotometer between the range of 200 nm to 400 nm and their spectra was overlaid. From the overlain spectra shown in **Figure 3** 263 nm was selected as analytical wavelength for Multi-component analysis using HPLC method.

METHOD VALIDATION

System Precision and System Suitability

The standard solution of 100µg/ml was prepared by diluting 1 ml of the standard solution to 10 ml with the mobile phase. It was injected six times into the HPLC system. The system suitability parameters were evaluated and found to be within the limits. The RSD for the peak areas from six replicate injections was calculated.

Linearity

The linearity of the method was demonstrated over the concentration range of 10-50 µg/ml for DAS and 5-25 µg/ml for PDH. A Series of dilutions were made by using the working standard solution (100 µg/ml). From the working standard solution 1.0, 2.0, 3.0, 4.0 and 5.0 ml for DAS and 0.5, 1.0, 1.5, 2.0 and 2.5 ml for PDH were pipetted out into 10 ml volumetric flasks and diluted with mobile phase and finally make up to the volume with mobile phase. The resulting solutions were labelled as 10, 20, 30, 40 and 50 µg /ml for DAS and 5, 10, 15, 20, and 25 µg /ml for PDH respectively. The linearity was calculated by the least square regression method.

Precision

The precision of an analytical method is the degree of agreement among the individual test results obtained when the method is applied repeatedly to multiple sampling of the same homogenous sample under prescribed conditions.

The precision of test procedure was evaluated by performing the six replicate injections of standard solution of 30 µg/ml and 15 µg/ml concentrations of DAS and PDH. The % relative standard deviation of DAS and PDH was found to be within the limits.

Accuracy (Recovery)

Drug accuracy was performed by spiking with equivalent amount of DAS and PDH raw material into each volumetric flask for each spike level to get the concentration equivalent to 50%, 100%, and 150% of the labelled amount of DAS and PDH as per the test method.

Limit of Detection (LOD)

The parameter LOD was determined on the basis of intercept and slope of the regression equation. It was calculated by using the following formula

$$\text{LOD} = 3.3 \times \text{S.D of y-intercepts} / \text{mean of slopes}$$

Table 1. System Suitability data of Pyridoxine Hydrochloride and Doxylamine Succinate

S. No	Concentration of PDH and DAS $\mu\text{g/ml}$	Pyridoxine Hydrochloride		Doxylamine Succinate	
		R_t	Peak Area	R_t	Peak Area
1	20	3.23	53481540	5.25	41813470
2	20	3.23	53485035	5.23	41502683
3	20	3.23	53214749	5.23	41945215
4	20	3.24	52463689	5.23	41698869
5	20	3.24	52931861	5.23	41473596
6	20	3.24	53511414	5.23	41844115
Mean		53181382		41712991	
SD		417056		191288.5	
%RSD		0.8		0.4	

Table 2. Summary of System Suitability Parameters

Parameters	Pyridoxine Hydrochloride	Doxylamine Succinate
Retention time (min)	3.24	5.23
Resolution (R_s)	1.99	
Tailing factor (t)	1.12	1.05
Theoretical plates (N)	11234	10357

Limit of Quantification (LOQ)

The parameter LOQ was determined on the basis of intercept and slope of the regression equation. It was calculated by using the following formula

$$\text{LOQ} = 10 \times \text{S.D of y-intercepts} / \text{mean of slopes}$$

Robustness

Robustness examines the effect of variation in operational parameters on the analysis results. For the determination of a method's robustness, parameters like variation in detector wavelength are varied within a realistic range and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range. It is the Measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides indication of its reliability during its normal usage.

Effect of Flow Rate Variation of HPLC method

Robustness of the method was checked by changing the flow rate from 0.8 ml/min and 1.2 ml/min instead of 1.0 ml/min by injecting of standard in 0.8 ml/min and 1.2 ml/min flow rate. The system suitability parameter of DAS and PDH standards are found within the limits. The method has been found to be robust from the flow rate 0.8 ml/min to 1.2 ml/min.

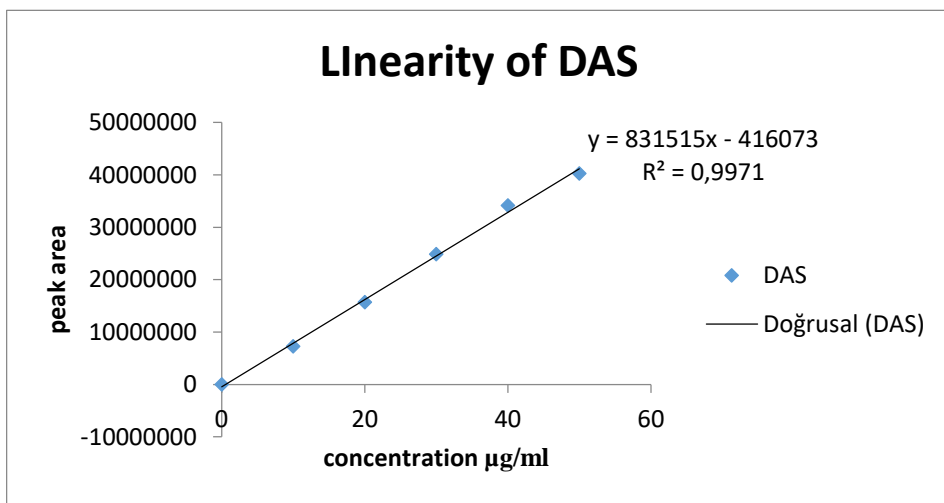


Figure 4. Calibration Curve of Doxylamine Succinate at 263nm by RP-HPLC method

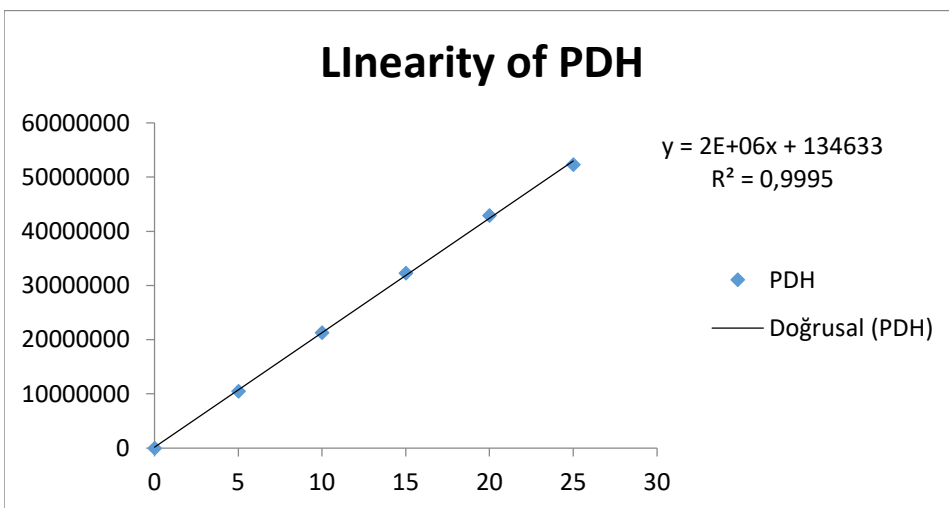


Figure 5. Calibration Curve of Pyridoxine Hydrochloride at 263nm by RP-HPLC method

Determination of DAS and PDH in Tablets

Weigh 20 tablets and crush them to powder. Weigh accurately tablet powder equivalent to 10mg of DAS and 10 mg of PDH were transferred in to 25ml volumetric flask, add 5ml of mobile phase, keep on shaking for 5 minutes. Sonicate for 10 minutes with occasional shaking in between. Make up the volume with mobile phase and mix well. A portion of the solution was filtered through a membrane filter of 0.22µm, discarding the first 1-2 ml. Pipette 0.3 ml from 25 ml volumetric flask and diluted to 10 ml with mobile phase. The amount of drug was calculated from the calibration curve.

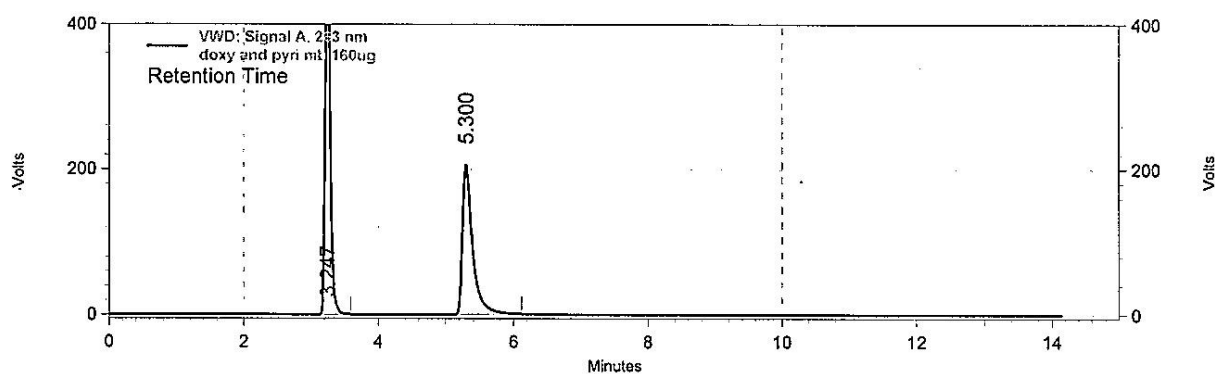


Figure 6. Optimized chromatogram of Doxylamine Succinate and Pyridoxine Hydrochloride

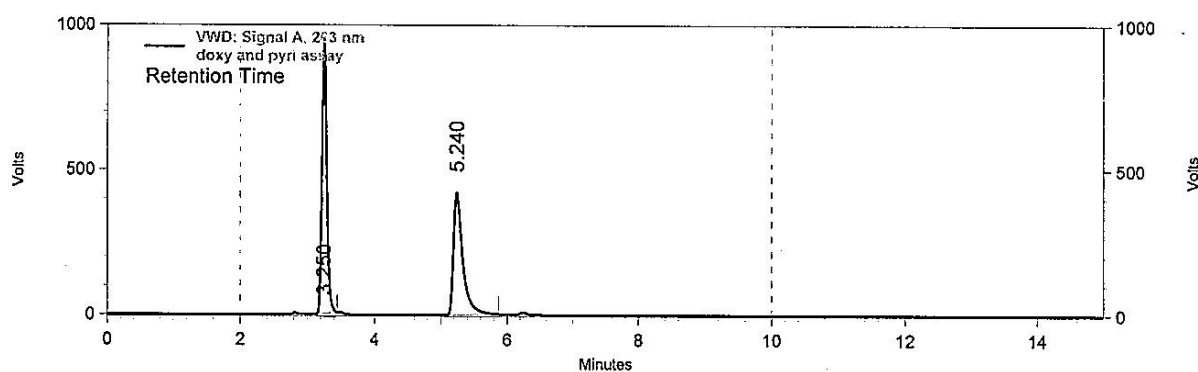


Figure 7. A typical chromatogram for assay of marketed formulation containing Doxylamine Succinate and Pyridoxine Hydrochloride

RESULTS AND DISCUSSION

After a number of trials with mobile phases of different composition, 20 mM potassium di hydrogen phosphate buffer (pH-5) and Methanol in the ratio of 40:60 v/v at a flow rate of 1.0 ml/min was selected as mobile phase because of better resolution and symmetric peaks. Doxylamine Succinate and Pyridoxine Hydrochloride were found to show appreciable absorbance at 263nm when determined spectrophotometrically and hence it was selected as the detection wavelength. An optimized chromatogram showing the separation of Doxylamine Succinate and Pyridoxine Hydrochloride at different R_{TS} of 3.24 and 5.30 minutes were shown in **Figure 2**.

System suitability was performed by injecting six replicate injections. The %RSD was found to be 0.4 for DAS and 0.8 for PDH. The results were shown in **Table 1**. The chromatograms confirm the presence of Doxylamine Succinate and Pyridoxine Hydrochloride at different R_{TS} of 3.24 and 5.30 minutes respectively without any interference. The linearity was observed in the concentration range of 10-50 $\mu\text{g/ml}$ for DAS and 5-25 $\mu\text{g/ml}$ for PDH, the R^2 values were found to be 0.997 and 0.999. The table with results was shown in **Table 2**. The linearity graph was shown as **Figure 4** and **5**. Both intra-day and inter-day precision was

Table 3. Linearity Data of Pyridoxine Hydrochloride and Doxylamine Succinate at 263 nm by RP–HPLC Method

S. No	Pyridoxine Hydrochloride			Doxylamine Succinate		
	Conc. (µg/mL)	R _t (min)	Peak Area	Conc. (µg/mL)	R _t (min)	Peak Area
0	0	0	0	0	0	0
1	5	3.2	10509106	10	5.3	7266448
2	10	3.2	21289761	20	5.3	15705171
3	15	3.2	32291833	30	5.3	24859598
4	20	3.2	42903437	40	5.3	34161460
5	25	3.2	52343646	50	5.3	40238177

Table 4. Determination of Precision for Pyridoxine Hydrochloride and Doxylamine Succinate with 30 µg/ml and 15 µg/ml concentrations of DAS and PDH by RP-HPLC method

S. No	Pyridoxine Hydrochloride		Doxylamine Succinate	
	Peak Areas			
	Intraday		Intraday	
1	52343646		42238177	
2	52501577		42178636	
3	52482375		42885191	
4	52088962		42802029	
5	51731723		42396872	
6	52804209		42529250	
MEAN	52325415		42505026	
SD	372458		290778	
%RSD	0.7		0.6	

performed by analysing 30 µg/ml for DAS and 15 µg/ml for PDH concentration. The %RSD values for both were not more than 2.0%. The results were shown in [Table 3](#). Accuracy of the method was verified by performing recovery studies by standard addition method. The percent recovery of the standard added to the pre-analysed sample was calculated. Accuracy was performed over the concentration range of 80%, 100% and 120% and mean recovery was found to be 98.2-100.8% for DAS and 98.6- 100.1% for PDH. The results were shown in [Table 4](#). The LOD values were found to be 1.8 and 0.4 µg/ml for DAS and PDH. The LOQ values were 4.4 and 4.2 µg/ml for DAS and PDH and respectively. Robustness was performed by analysing the samples at different wavelengths and varying flow rates. The method was found to be robust after changing the conditions like detection wavelength (± 2 nm) and flow rate (± 0.2 ml). %RSD was calculated for each variation and reported. The results were shown in [Table 5](#). The method was found to be specific for the combination of interest after verifying the chromatograms showing no interference of the excipients present. Hence, the method was well suitable for the estimation of the commercial formulations of the selected combination. The typical chromatogram for assay of marketed formulations was shown in [Figure 7](#).

Table 5. Determination of Accuracy of Pyridoxine Hydrochloride and Doxylamine Succinate

Recovery level	Amount of Standard drug added ($\mu\text{g/mL}$)		Amount of test drug added ($\mu\text{g/mL}$)		Total amount of drug recovered ($\mu\text{g/mL}$)		% Recovery (w/w)	
	PYRI	DOX	PYRI	DOX	PYRI	DOX	PYRI	DOX
80%	5	10	20	40	25.2	50.09	100.8	100.1
100%	15	30	20	40	34.5	69.2	98.5	98.8
120%	25	50	20	40	44.2	88.8	98.2	98.6

Table 6. Robustness of DAS and PDH variable flow rates (30 $\mu\text{g/ml}$ and 15 $\mu\text{g/ml}$)

Flow rate (ml/min)	DAS		PDH	
	RT (min)	Peak Area	RT (min)	Peak Area
0.8	5.4	21289761	3.4	15705171
1.2	5.1	21289761	3.1	15705171

Table 7. Robustness of DAS and PDH variable wavelengths (30 $\mu\text{g/ml}$ and 15 $\mu\text{g/ml}$)

Flow rate (ml/min)	DAS		PDH	
	RT (min)	Peak Area	RT (min)	Peak Area
260	5.3	21214032	3.2	15625344
265	5.3	21223242	3.2	15203417

CONCLUSION

The RP-HPLC method developed and validated allows a simple and fast quantitative determination of Doxylamine Succinate and Pyridoxine Hydrochloride. The developed RP-HPLC method was validated according to ICH guidelines and was found to be applicable for the routine analysis of Doxylamine Succinate and Pyridoxine Hydrochloride in their single and combined dosage forms.

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