ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF PYRIDOXAMINE DIHYDROCHLORIDE AND ACETYLCYSTEINE IN TABLET DOSAGE FORM.

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ABSTRACT

The present study describes simple, accurate, precise UV spectrophotometric method for the simultaneous estimation of Pyridoxamine dihydrochloride and Acetylcysteine in tablet dosage form. The method involved measurement of absorbance at two wavelengths 220 nm and 216 nm λmax of Pyridoxamine dihydrochloride and Acetylcysteine. Linearity was observed in the range of 2.5microgram/ml to 12.5microgram/ml (r^2=0.997) for Pyridoxamine dihydrochloride and 15microgram/ml to 75microgram/ml (r^2=0.999) for Acetylcysteine. The percentange mean recovery was found to be 99.26% for Pyridoxamine dihydrochloride and 100.7 % for Acetylcysteine. The percentage RSD for the recovery study was less than 2. The methods were validated as per ICH guidelines.

Keywords: Pyridoxamine dihydrochloride, Acetylcysteine, Simultaneous equation, validation, UV spectrophotometer.

INTRODUCTION

Pyridoxamine dihydrochloride chemical name is 4-(aminomethyl)-5-(hydroxymethyl)-2-methylpyridin-3-ol dihydrochloride. Pyaridoxine, pyaridoxal and pyridoxamine are different forms of vitamin B6 that undergo phosphorylation to produce pyridoxal 5-phosphate (PLP). PLP is the co-factor for a large number of enzymes involved in the metabolism of amino acids. Vitamin B6 is available in most food.

Although vitamin B6 supplements have become popular in the treatment of nausea in pregnancy, carpal tunnel syndrome and pre-menstrual syndrome,there is no convincing evidence of benefit [1-3].
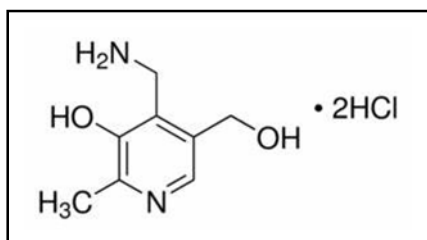
Figure 1: Structure of Pyridoxamine dihydrochloride

Acetylcysteine is also known as (N-Acetylcysteine or N-acetyl-L-cysteine or NAC) is derived from cysteine by attaching an acetyl group to amino group [4]. N-Acetylcysteine is an active pharmaceutical agent and nutritional supplement primarily used as a mucolytic agent and in the management of paracetamol overdose. Acetylcysteine it is an antioxidant in its own right but is also deacetylated to cysteine, which participates in the synthesis of the antioxidant glutathione. If you have an allergy to Acetylcysteine or any other part of Acetylcysteine solution.
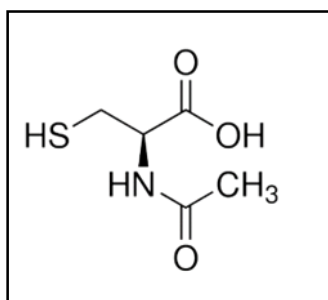
Figure 2: Structure of Acetylcysteine

From literature survey it was found that no any UV method has been reported on this combination respectively. In this present research work, it was proposed to developed and validate a new, simple, and accurate UV method for simultaneous estimation of Pyridoxamine dihydrochloride and Acetylcysteine in marketed dosage formulations.

In the present work, simultaneous estimation of Pyridoxamine dihydrochloride and Acetylcysteine in combined dosage form is developed and validated as per ICH guideline[5].

MATERIAL AND METHOD:

Chemicals and Reagents:

Analytical pure sample of Pyridoxamine dihydrochloride and Acetylcysteine were received as a gift sample from Cipla Private Limited were used in the study. The pharmaceutical dosage form used in this

study was NEFROSAVE FORTE labeled to contain Acetylcysteine and Pyridoxamine dihydrochloride 300/50 mg per tablet. The solvent used were of Methanol and Distilled water used in preparation of mobile phase.

Selection of wavelength:

UV spectra of Pyridoxamine dihydrochloride and Acetylcysteine at 220 nm and 216 nm respectively. Mobile phase Methanol: Water (50:50%v/v) is used for this good peaks, good absorbance and better sensitivity. Both drugs absorbed at same point shown in figure 3.
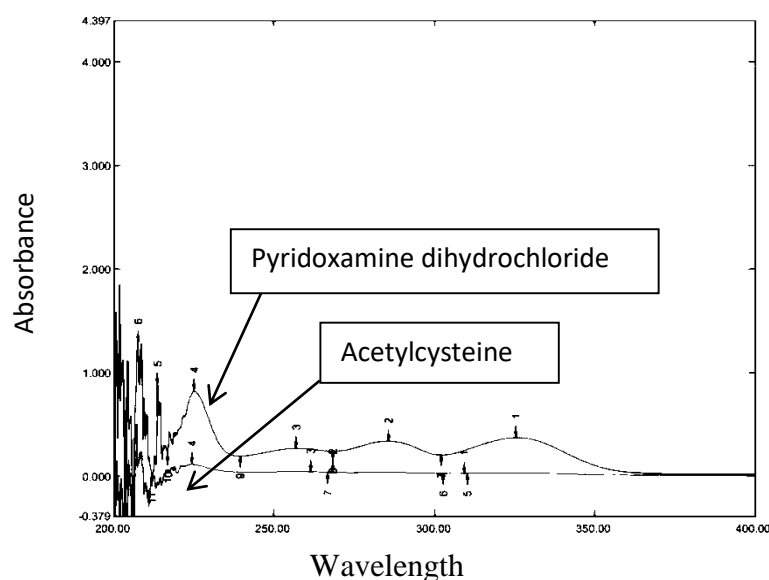
Figure 3: Uv Spectra of Pyridoxamine dihydrochloride & Acetylcysteine

Instrumentation:

A shimadzu 1800UV/VIS double beam spectrophotometer with 1 cm matched quartz cells was used for all spectral measurements [6].

Preparation of Mobile phase:

1000 ml mobile phase was prepared by mixing 50 ml methanol and 50 ml distilled water (50:50%v/v).

Preparation of stock solution of Pyridoxamine dihydrochloride:

Prepare a standard stock solution of Pyrodoxamine dihydrochloride by adding 50 mg in 50 ml volumetric flask & make the volume to 50 ml with diluent. Then pipet out 0.1ml and add 10ml volumetric flask and make the volume again 10ml with diluent. (conc. of Pyridoxamine dihydrochloride = 10 microgram/ml).

Acetylcysteine:

Prepare a standard stock solution of Acetylcysteine by adding 100 mg in 100 ml volumetric flask & make the volume to 100 ml with diluent. Then pipet out 0.6 ml and add 10 ml volumetric flask and make the volume again 10ml with diluent. (conc. of Acetylcysteine = 60 microgram/ml).

Simultaneous estimation of Pyridoxamine Dihydrochloride and Acetylcysteine:

In simultaneous method we used Absorbances at two selected wavelengths. To determine the λmax of both the drugs we scan in the range of 200-400 nm. Standard solutions of different concentrations of both drugs were prepared in mobile phase. Absorbance of Pyridoxamine dihydrochloride (10microgram/ml) and Acetylcysteine (60 microgram/ml) were recorded at two wavelenghts 220 nm and 216 nm by using simultaneous equation method [7-9].

$$Cx = A2ay1 - A1ay2/ax2ay1 - ax1ay2$$

$$Cy = A1ax2 - A2ax1/ax2ay1 - ax1ay1$$

Cx = concentration of Acetylcysteine

Cy = concentration of Pyridoxamine dihydrochloride

ax1and ax2 = absorptivity value of Acetylcysteine at 216nm and 220nm

ay1 and ay2 = absorptivity value of Pyridoxamine dihydrochloride at 216nm and 220nm

A1 = absorbance of standard mixture at 216 nm

A2 = absorbance of standard mixture at 220 nm

Analysis of marketed formulation:

Five tablets of brand name NEFROSAVE FORTE were used. From the five tablets accurately weighed the powder equivalent to single tablet (Pyridoxamine dihydrochloride 50mg and Acetylcysteine 300mg), then transferred to 50 ml volumetric flask to this methanol was added and for dissolving the drug used sonicator approximately for 10 min. then passed it through the whatman filter paper and make up volume up to 50 ml from diluent. From this solution made a 10microgram/ml and 60microgram/ml solution for Pyridoxamine dihydrochloride and Acetylcysteine resepectively [10-15].

Table 1: Analysis of marketed formulation

Sr no.	Pyridoxamine dihydrochloride			Acetylcysteine		
	Absorbance	Amount recovered (microgram/ml)	% Recovery	Absorbance	Amount recovered (microgram/ml)	% Recovery
1	0.290	9.76	97.6	0.333	61.09	101.8
2	0.293	9.86	98.6	0.336	61.42	102.3
3	0.295	9.93	99.3	0.332	60.77	101.2
4	0.297	10	100	0.339	61.26	102.1
5	0.294	9.9	99	0.341	60.93	101.5
Mean	0.293	9.89	98.9	0.336	61.094	101.8
% RSD	0.8810	0.8987	0.8987	1.1404	0.4218	0.4218

Method validation [16-17]:

Validation of an analytical method is the process to establish that the performance characteristics of the developed method meet the requirements of the intended analytical application. The UV method was validated in terms of linearity, accuracy, precision, LOD and LOQ.

Linearity:

Linearity was studied by plotting a graph of absorbance is directly propotional to the concentration. A series of standard solution of Pyridoxamine dihydrochloride were prepared in the concentration range of about 2.5microgram/ml to 12.5 microgram/ml and Acetylcysteine concentration range is 15 µg/ml to 75 µg /ml is shown in below table (2). Linearity graph of Pyridoxamine dihydrochloride and Acetylcysteine shown in fig.no.4 & 5.

Table 2: Linearity study of Pyridoxamine Dihydrochloride and Acetylcysteine

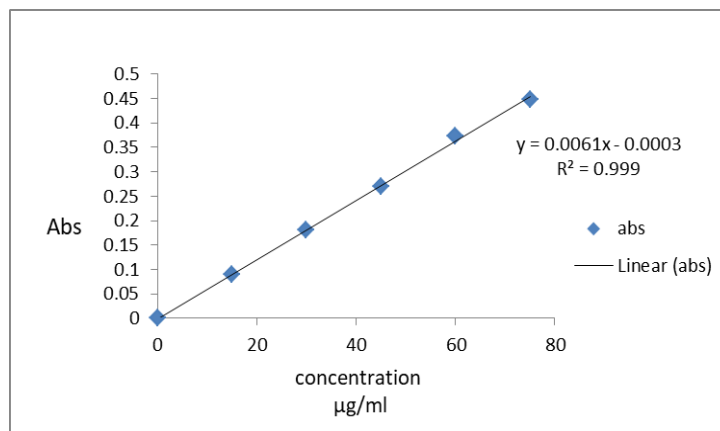| Sr no.	Concentration (microgram/ml)		Absorbance	
	Pyridoxamine dihydrochloride	Acetylcysteine	Pyridoxamine dihydrochloride at 220nm	Acetylcysteine at 216 nm
1	2.5	15	0.072	0.090
2	5	30	0.159	0.180
3	7.5	45	0.230	0.269
4	10	60	0.290	0.373
5	12.5	75	0.370	0.448
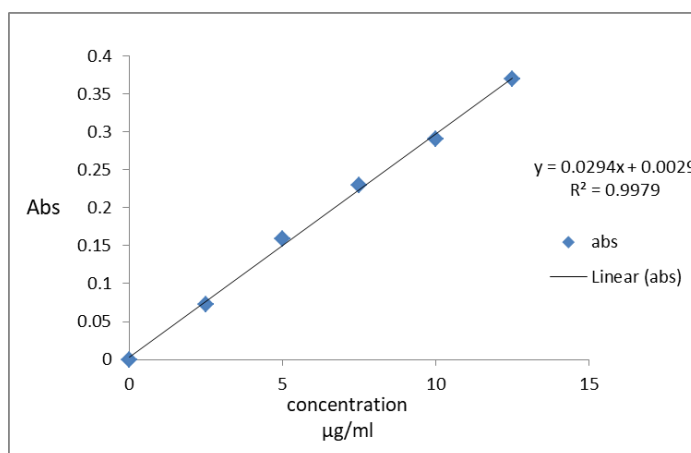
Fig 4: linearity graph of Pyridoxamine dihydrochloride

Fig 5: linearity graph of Acetylcysteine

Precision:

Repeatability measurement were carried out by analyzing 6 different solutions containing concentration 5, 7.5, 10 μg/ml of Pyridoxamine dihydrochloride and 30, 45, 60microgram/ml of Acetylcysteine. For determination of intra-day and inter-day variation the absorbance were measured three times in the days. Result of % RSD was found to be below 2 shown in below tables (3,4,5,6).

Table 3: Intra-day precision of Pyridoxamine dihydrochloride

Conc. microgram/ml	Absorbance			Mean Absorbance	SD	%RSD
	Trial 1	Trial 2	Trial 3			
5	0.164	0.166	0.172	0.717	0.003606	0.5028
7.5	0.235	0.225	0.239	0.725	0.003055	0.4213
10	0.312	0.316	0.318	0.815	0.003055	0.3748

Table 4: Intra-day precision of Acetylcysteine

Conc. microgram/ml	Absorbance			Mean Absorbance	SD	%RSD
	Trial 1	Trial 2	Trial 3			
30	0.182	0.184	0.185	0.738	0.007839	1.0621
45	0.265	0.270	0.269	0.741	0.007839	1.0578
60	0.375	0.373	0.378	0.753	0.007737	1.0275

Table 5: Inter-day precision of Pyridoxamine dihydrochloride

Conc. microgram/ml	Absorbance			Mean Absorbance	SD	%RSD
	Trial 1	Trial 2	Trial 3			
5	0.165	0.167	0.173	0.709	0.007435	1.0486
7.5	0.232	0.226	0.238	0.712	0.007396	1.0387
10	0.314	0.315	0.317	0.724	0.002517	0.3475

Table 6:Inter-day precision of Acetylcysteine

Conc. microgram/ml	Absorbance			Mean Absorbance	SD	%RSD
	Trial 1	Trial 2	Trial 3			
30	0.184	0.183	0.186	0.184	0.001527	0.8301
45	0.267	0.269	0.271	0.269	0.002	0.7434
60	0.371	0.370	0.376	0.372	0.003214	0.8641

Accuracy:

This parameter is performed to determine the closeness of the test results with that of the true value which is expressed as % recovery. These studies were performed at three different levels (50% ,100% , and 150%) and the % recovery of Pyridoxamine dihydrochloride and Acetylcysteine was calculated below table (7& 8).

Table 7: Recovery study of Pyridoxamine dihydrochloride

Level	Conc.(microgram/ml)		Absorbance	% Recovery	Mean % recovery ±RSD
	Sample	Standard			
50%	10	5	0.434	99.26	99.73±0.4712
			0.438	100.2	
			0.436	99.73	
100%	10	10	0.564	96.85	96.53±0.3370
			0.562	96.55	
			0.560	96.2	
150%	10	15	0.722	99.28	99.56±0.2812
			0.724	99.56	
			0.726	99.84	

Table 8: Recovery study of Acetylcysteine

Level	Conc.(microgram/ml)		Absorbance	% Recovery	Mean % recovery ±RSD
	Sample	Standard			
50%	15	7.5	0.139	100.7	100.7±0.6951
			0.140	101.4	
			0.138	100	
100%	15	15	0.182	99.43	100.3±0.8430
			0.184	100.5	
			0.185	101.1	
150%	15	22.5	0.226	99.09	99.25±0.6893
			0.228	100	
			0.225	98.66	

Robustness:

The robustness of the analytical method is measure of its capacity to remain unaffected by small but deliberate variations in the method parameters and provides an indication of its reliability during normal usage. The robustness of the method was studied for Pyridoxamine dihydrochloride and Acetylcysteine.

Sensitivity:

The limit of detection [LOD] and limit of quantitation [LOQ] parameters were calculated using following equations; LOD $=3.3\sigma/S$ and LOQ $= 10\ \sigma/S$

Where, σ = standard deviation of y-intercept of regression line.

 S= slope of the calibration curve.

Limit of Detection (LOD) and Limit of Quantitation (LOQ) Determination:

Limit of quantitation is 3 times more than the limit of detection resp. The LOD value of Pyridoxamine dihydrochloride and Acetylcysteine is 4.60 microgram/ml and 19.78 microgram/ml respectively and the LOQ value were found to be 13.96 microgram/ml and 59.95 microgram/ml Pyridoxamine dihydrochloride and Acetylcysteine.

Table 9: Result of LOD AND LOQ

Sr no.	Name of drugs	LOD (microgram/ml)	LOQ (microgram/ml)
1	Pyrodoxamine dihydrochloride	4.60	13.96
2	Acetylcysteine	19.78	59.95

RESULT AND DISCUSSION:

The proposed method is based on spectrophotometric simultaneous estimation of Pyridoxamine dihydrochloride and Acetylcysteine in this method methanol and distilled water is used as solvent. the calibration plot for the method was linearity range concentration of 15 to 75microgram/ml for Acetylcysteine and 2.5 to 12.5microgram/ml for Pyridoxamine dihydrochloride respectively. The determination of coefficients (r^2) was 0.999 and 0.997 for Acetylcysteine and Pyridoxamine dihydrochloride respectively. The method was found to be precise and as the %RSD values for intra-day and inter-day were found to be less than 2% for Acetylcysteine and Pyridoxamine dihydrochloride respectively. The LOD and LOQ were found to be 19.78 microgram/ml and 59.95 microgram/ml for Acetylcysteine and 4.6 0 microgram/ml and 13.96 microgram/ml for Pyridoxamine dihydrochloride respectively. The percentange mean recovery was found to be 99.26% for Pyridoxamine dihydrochloride and 100.7 % for Acetylcysteine. The results of assay showed that the amount of drug as indicated by % assay for 101.8 % Acetylcysteine and 98.9 % for Pyridoxamine dihydrochloride. The proposed method was also successfully applied to a pharmaceutical formulation[18-19].

CONCLUSION:

The results of our study indicate that the proposed UV spectroscopic method is simple, rapid, precise, and accurate. The developed UV spectroscopic methods were found suitable for determination of Pyridoxamine dihydrochloride and Acetylcysteine as bulk drug and in marketed solid dosage formulation without any interference from the excipients. Statistical analysis proves that, these methods are repeatable and selective for the analysis of Pyridoxamine dihydrochloride and Acetylcysteine.

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