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METHOD DEVELOPMENT AND VALIDATION OF FOLIC ACID BY UV-VISIBLE SPECTROSCOPY

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ABSTRACT: The present study a simple, accurate, precise and cost effective UV-Visible spectrophotometric method for development and validation of folic acid as vitamin. folic acid is a water soluble vitamin, so solvent used throughout the experiment was chloroform, butanol,0.1N NaoH, water .the absorption maxima of drug was found at 250nm . Beer's law was obeyed in the range of 5ppm-30ppm. The developed method was successfully validated with respect to linearity, accuracy and precision. The method was validated and shown linearity in mentioned concentration. The percentage relative standard deviation of inter-day and intra-day precision range 45.15%. Hence proposed method was precise, accurate and cost effective, simple and rapid. This validated method can be applicable for quantitative determination of the titled drug with respect to assay for solid dosage form.

INTRODUCTION: The Vitamin (B₆) folic acid is water soluble Vitamin.



FOLIC ACID

IUPAC Name:

(25)-2-[(4-{[(2-amino - 4 - hydroxypteridin - 6 - yl)methyl]amino}phenyl)formamido]pentanidionic acid.

QUICK RESPONSE CODE				
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Molecular Formula: C₁₉H₁₉O₆

Molecular Weight: 441.4

Method Development:

Solubility:

Soluble in water, chloroform, Butanol, 0.1N NaOH. And practically insoluble in NaOH, methanol, ether.

Preparation of Stock Solution:

Standard stock solution of folic acid was prepared by dissolving 1.6gm of folic acid in 100ml water which gives 100ppm concentration.

Preparation of Working Standard:

From the above stock solution 1ml pipetted in to 10ml volumetric flask and the volume was made up with water to produce concentration of 10ppm. The solution was scanned in UV-Visible spectrophotometer in the range 600-400nm using water as a blank. The wavelength corresponding to masximum absorbance (λ max) was found as a 250nm.

Method Validation:

Linearity: Various aliquots were prepared from the secondary stock solution (100ppm) ranging from 10ppm-50ppm. The samples were scanned in UV-Visible Spectrophotometer against water as blank. It was found that the selected drug shows linearity between the ranges of 10ppm in Fig.1.



Accuracy:

Solutions were prepared in triplicate at levels 80%, 100% and 120% of test concentration using folic acid working standard as per the test method and taken absorbance of each solution in triplicate. The recovery results showed that the proposed method has an acceptable level of accuracy for

Table 1: ACCURACY

folic acid which is from 80%-120% of test concentration is 99.51% -100.01% in Table 1.

Precision:

Precision of the method was demonstrated by intraday and inter-day variation studies. In intra-day variation study nine different solutions of same concentration 10ppm, 20ppm, 30ppm, were analyzed three times in a day i.e. morning, afternoon and evening and the absorbance is noted. From the absorbance result mean, standard deviation and %RSD was calculated and given in following table. In the inter-day variation studies, solution of same concentration 10ppm, 20ppm, 30ppm, 40ppm, 50ppm.were analyzed three times for the three consecutive days and the absorbance result mean, standard deviation and %RSD was calculated and given in following Table 2.

Ruggedness:

Ruggedness of the method was determined by carrying out the analysis by different analyst and the respective absorbance of 20ppm was noted. The result was indicated as %RSD and given in Table 3.

RESULT AND DISCUSSION:

The performed experimental work it showed that the result obtained are satisfactory. The Accuracy; Linearity, ruggedness and Precision test of vitamin folic acid are 99% accurate result.

1; A	CUNACI						
	Concentration	Formulation	Pure Drug	%Recovery	Mean	SD	%RSD
	(%)	(ppm)					
	80	10	8	0.347			
	80	10	8	0.043	0.442	0.135	0.67
	80	10	8	0.553			
	100	10	10	0.428			
	100	10	10	0.335	0.626	0.255	1.27
	100	10	10	1.115			
	120	10	12	0.507			
	120	10	12	0.810	0.915	0.388	1.94
_	120	10	12	1.430			

TABLE 2: PRECISION

TABLE 2: PRECISION			 TABLE 3: RUGGEDNESS		
Concentration	Absorbance	%C.V	ANALYST 1: CHEMITO		
(ppm)			Concentration	Absorbance	Stastical
10	0.188	274.34	(ppm)		Analysis
20	0.423	74.12	10	0.333	Mean 0.333
20	0.423	74.12	10	0.334	%RSD 0.99
30	0.720	45.15	10	0332	

ANALYST: 2(SHIMADZU)	
TABLE 4: SUMMARY OF VALIDATION PARAMETERS OF	F
SIMPLE UV SPECTROSCOPY	

Concentration	Absorbance	Stastical Analysis	e Sta	
(ppm)				
10	0.294	Mean 0.293]	
10	0.293	%RSD 1.00		
10	0.292			
Sr.No	Param	eter Result	ameter	
1	λma	x 250nm	max	
2	Accura	acy 1.27%	curacy	
3	Precis	ion 45.15%	cision	

Ruggedness

0.33%

CONCLUSION: The method reported above is simple, fast, and suitable for analysis of watersoluble vitamins. unlike The the gas chromatographic and HPLC procedures, the instrument is simple and affordable. The importance lies in the chemical reactions upon which the procedures are based rather than upon the sophistication of the instrument. This aspect of spectrophotometric analysis is of major interest in analytical pharmacy since it offers distinct possibility in the assay of a particular component. The reagents utilized in the proposed methods are cheap, readily available and the procedure does not involve any critical reaction conditions or tedious sample preparation. The method is unaffected by slight variations in experimental conditions such as reagent concentration, temperature. The wide applicability of the new procedure for routine quality control is well established by the assay of vitamin B_6 (Folic acid). Thus, the proposed validated method can be use for quality control of title material as well as its formulation.

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