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RP-HPLC Method Development and Validation for Estimation of Metadoxine in API and Pharmaceutical Dosage form

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ABSTRACT

A rapid, specific, accurate and precise reverse phase high performance liquid chromatographic method has been developed and validated for Metadoxine, in its pure form as well as in tablet dosage form. Chromatography was carried out on a cap cell pack C18 (250 x 4.6mm, 5µm) column using a mixture of ACN: Water (65:35% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 305nm. The Retention time of the Metadoxine was 3.155±0.02min respectively. The method produce linear responses in the concentration range of 10-50µg/ml of Metadoxine. The precision of the method was demonstrated with %RSD values of below 2% while the % recovery was found in between 98102%. There is no interference of any compounds present in pharmaceutical dosage form was observed. According to the validation results the proposed method was found to be rapid, simple, specific, accurate, precise and robust. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Metadoxine, RP-HPLC, validation.

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CONTENTS

1. Introduction

Chemical name/ Nomenclature / IUPAC Name: L-Proline, 5-oxo-, compd. with 5- hydroxy-6-methylpyridine-3,4-dimethanol
Molecular Formula: $13H18N2O6$
Molecular Weight: 298.29 g/mol.
Official Pharmacopoeia: USP
Physicochemical Properties:
Description (Physical State): Solid
Solubility: Water Solubility 1.28 mg/ml
Storage Conditions: Store it at 15 - 30 degree C. Protect from moisture and heat.
Dosage: Tablet 500 mg

Melting point: 122 °C
pKa (strongest acidic): 13.14
Log P: 2.3

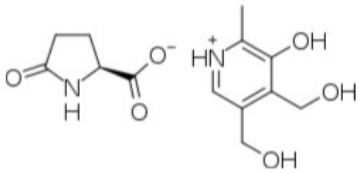

Figure 1: Metadoxine

Pharmacokinetic properties:
Half-life:9.2 (+/- 4.8) hours
Absorption: The absolute bioavailability of metaxalone from Skelaxin tablets is not known.
Volume of Distribution: 800 L
Metabolism: Probably hepatic.
Excretion: Metaxalone is metabolized by the liver and excreted in the urine as unidentified metabolites.
Adverse effects/Side effects: Sleepiness, Numbness, Skin Rash, and Loose Motion

Pharmacodynamics:
Metaxalone is a skeletal muscle relaxant indicated as an adjunct to rest, physical therapy, and other measures for the relief of discomforts associated with acute, painful musculoskeletal conditions. The mode of action of this drug has not been clearly identified, but may be related to its sedative properties. Metaxalone does not directly relax tense skeletal muscles in man.

2. Material and Methods

Table 1: List of Instruments used

Name of the instrument	model	Make
HPLC	LC 100	Shimadzu
Weighing balance	Gold -300p	Phoenix
UV- vis specrophotometer	UV-3000	Lab india
Digital PH meter	DI-45P	DATAL
Ultra sonic cleaner	CD4820	LIFECARE
Micro filtration unit	VE115N	Value

Table 2: List of chemicals used

S.No	Chemical	Brand names
1	Metadoxine(Pure)	Hetro labs
2	Water and Methanol for HPLC	CDH Pvt India
3	Acetonitrile for HPLC	CDH Pvt India

Preparation of standard solution:
Accurately weigh and transfer 10 mg of Metadoxine 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 0.3ml of the above Metadoxine 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 ACN: Water with varying proportions. Finally, the mobile phase was optimized to ACN: Water (65:35% v/v) respectively.

Optimization of Column:
The method was performed with various C18 columns like Symmetry, Zodiac, Xterra. Cap cell pack C18 (4.6 x 150mm, 5μm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions:
Instrument used: LC 100 PDA detector
Temperature: 35ºC
Column: cap cell pack C18 (250 x 4.6mm, 5□m)
Mobile phase: ACN: Water (65:35% v/v) Flow rate : 1.0mL/min
Wavelength: 305 nm
Run time: 8 minutes

Method validation

Preparation of mobile phase:

Preparation of mobile phase:
Accurately measured 650 ml (65%) of HPLC Methanol and 350 ml of Water (35%) were mixed and degassed in a 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 diluents.

Validation Parameters

System Suitability
Accurately weigh and transfer 10 mg of Metadoxine working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.3ml of the above Metadoxine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Specificity study of drug:

Preparation of Standard Solution:
Accurately weigh and transfer 10 mg of Metadoxine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 0.3ml of the

above Metadoxine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Preparation of Sample Solution:

Take average weight of the Powder and weight 10 mg equivalent weight of Metadoxine sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.3ml of the above Metadoxine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

Preparation of Drug Solutions for Linearity:

Accurately weigh and transfer 10 mg of Metadoxine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

3. Results and Discussion

Trail 1:

Column: cap cell packC18 (250× 4.6mm5µm)
Column temperature: 30ºC
Wave length : 305 nm
Mobile phase ratio: Methanol: water (50:50% v/v) Flow rate: 1.0mL/min
Injection volume: 10 µl
Run time: 9minutes

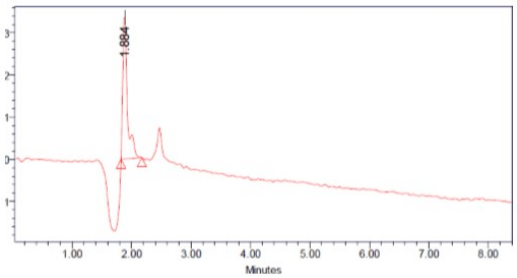

Figure 2: Chromatogram for Trail 1

Trail 2:

Column : cap cell pack C18 (250 x 4.6mm, 5□m)
Column temperature: 30ºC
Wavelength: 305 nm
Mobile phase ratio: Methanol: Water (70:30)
Flow rate: 1.0mL/min
Injection volume: 10 µl
Run time: 7 minutes

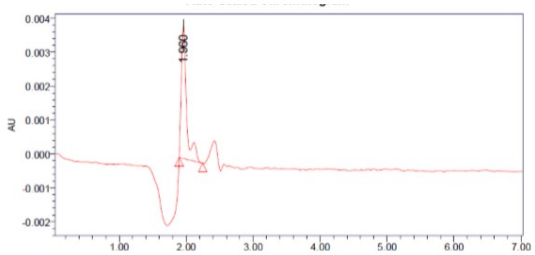

Figure 3: Chromatogram for trail 2

Optimized Chromatogram (Standard)

Column: cap cell pack C18 (250 x 4.6 mm, 5□m)
Column temperature : 35ºC
Wavelength: 305 nm
Mobile phase ratio: ACN: Water (65:35% v/v) Flow rate : 1.0mL/min
Injection volume: 10 µl
Run time: 8 minutes

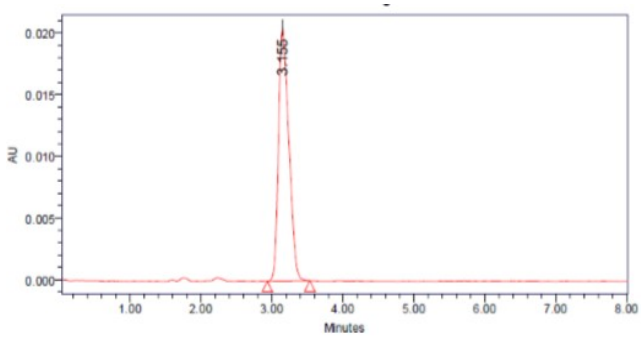

Figure 4: Optimized Chromatogram (Standard)

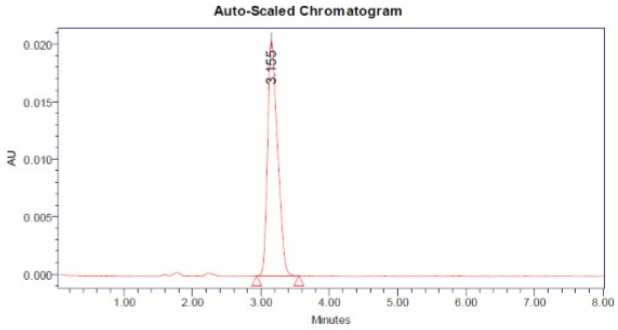

Figure 5: Optimized Chromatogram (Sample)

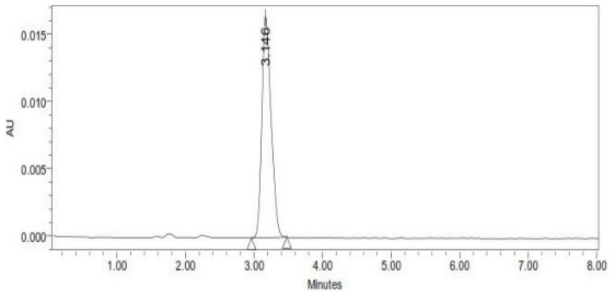

Figure 6: Chromatogram showing injection -1 (standard)

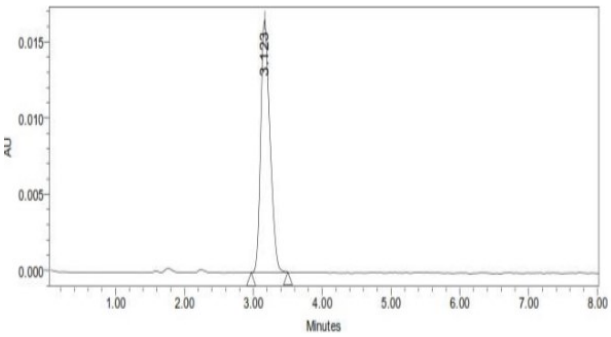

Figure 7: Chromatogram showing injection -2 (standard)

Table 3: Results of Assay (Standard) for Metadoxine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Metadoxine	3.146	153885	16537	6533	1.3
2	Metadoxine	3.123	153763	16521	5973	1.3
3	Metadoxine	3.192	152764	16537	5163	1.3
4	Metadoxine	3.164	153975	16427	5082	1.3
5	Metadoxine	3.181	153975	16573	5726	1.3
Mean			153672.4			
Std. Dev.			515.2017			
% RSD			0.33526			

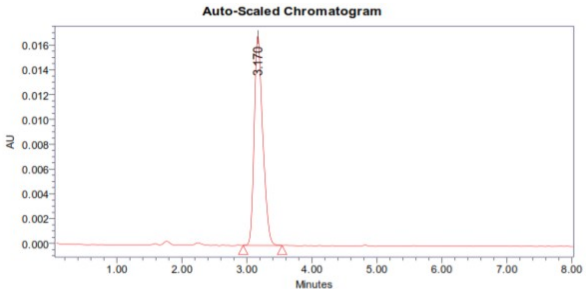

Figure 8: Chromatogram showing assay of sample injection

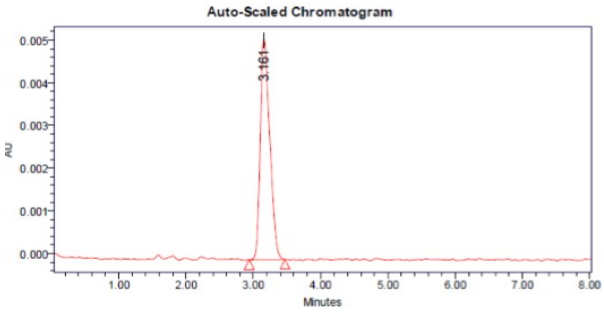

Figure 9: Chromatogram showing linearity level-1

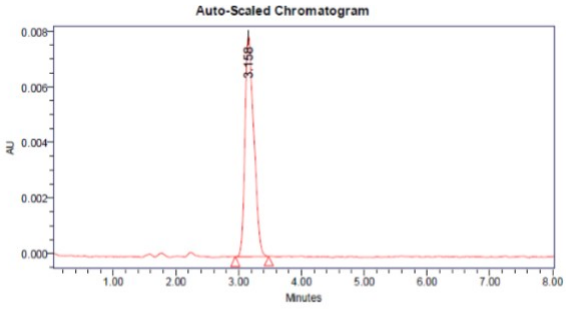

Figure 10: Chromatogram showing linearity level-2

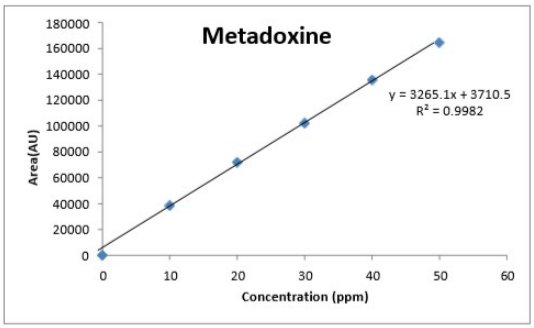

Figure 11: Linearity plot

Validation Criteria:

The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 3710. These values meet the validation criteria.

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability

Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

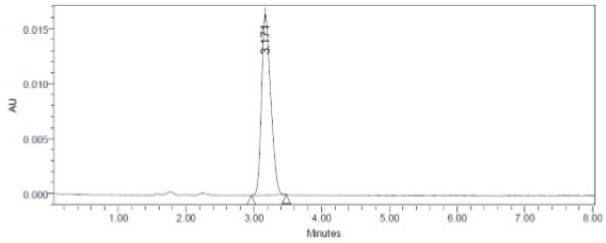

Figure 12: Chromatogram showing precision injection -1

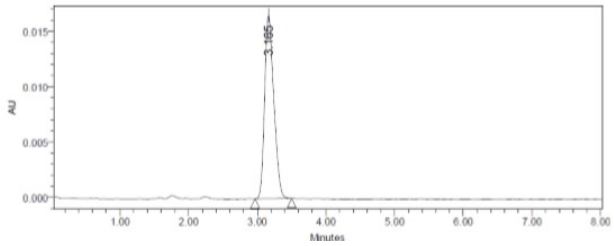

Figure 13: Chromatogram showing precision injection -2

Table 4: Results of method precision for Metadoxine

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Metadoxine	3.165	153488	16579	5510.1	1.3
2	Metadoxine	3.163	153650	16048	5255.1	1.3
3	Metadoxine	3.158	153852	16033	5174.0	1.3
4	Metadoxine	3.167	154083	16324	4352.7	1.3
5	Metadoxine	3.171	154342	16554	5438.0	1.3

Mean: 153882.8, Std.dev: 339.9, %RSD: 0.2

Table 5: The accuracy results for Metadoxine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	53261.67	15	14.9	99.3	99.4%
100%	103318	30	29.87	99.5	
150%	151061.7	45	44.79	99.5	

Table 6: Results of ruggedness for Metadoxine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Metadoxine	3.165	153488	16579	6510.1	1.3
2	Metadoxine	3.163	153650	16048	2255.1	1.3
3	Metadoxine	30158	153852	16033	5174.0	1.3
4	Metadoxine	3.167	154083	16324	5352.7	1.3
5	Metadoxine	3.171	154342	16554	5438.0	1.3
6	Metadoxine	3.171	154342	16554	5438.0	1.3
Mean			153882.8			
Std. Dev.			339.9			
% RSD			0.2			

Limit of Detection for Metadoxine

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD= 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

= 3.3 × 1314.685 /3265

= 1.3µg/ml

Limit of Quantitation for Metadoxine

The quantization limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined. LOQ=10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

=10 × 1314.685 /3265

= 4.0µg/ml

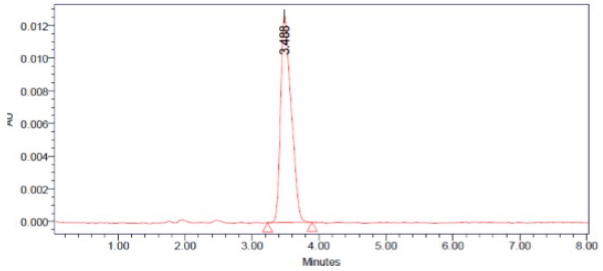

Figure 14: Chromatogram showing less flow of 0.9ml/min

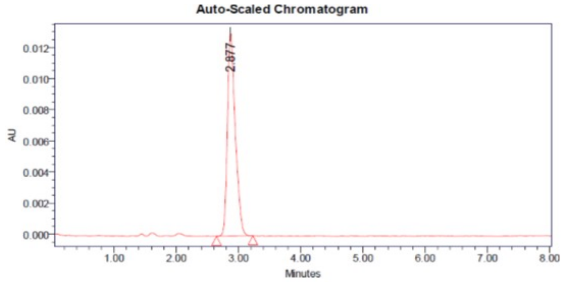

Figure 15: Chromatogram showing more flow of 1.1 ml/min

4. Conclusion

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for quantitative estimation of Metadoxine in bulk drug and pharmaceutical dosage form. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Water Metadoxine was freely soluble in ethanol, methanol and sparingly soluble in ACN: Water 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 Metadoxine bulk drug and in Pharmaceutical dosage forms.

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