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# DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF METADOXINE

**P. Aravinda Reddy[1] and Ramya Sri. S[2]**

**[1] Department of Pharmaceutical analysis, Vaageswari College of Pharmacy, Karimnagar, Telangana 505527, India**

**[2]Department of Pharmacy, University College of Technology, Osmania University, Hyderabad, Telangana, 500007, India**

***Corresponding Author Email: surapharmalabs1@gmail.com**

## ABSTRACT

*A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validation of Metadoxine, in its pure form as well as in tablet dosage form. Chromatography was carried out on an ODS C18 (4.6 x 150mm, 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 produces linear responses in the concentration range of 10-50µg/ml of Metadoxine. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.*

## KEY WORDS

*Metadoxine, RP-HPLC, validation.*

## INTRODUCTION

### Analytical chemistry[1]

Analytical chemistry is a scientific discipline used to study the chemical composition, structure, and behavior of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative, and structural information on the nature of matter.

- **Qualitative analysis** is the identification of elements, species and/or compounds present in sample.
- **Quantitative analysis** is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups). An element, species or compound that is the subject of analysis is known as analyte. The remainder of the material or sample of which the analyte(s) form(s) a part is known as the matrix.

The gathering and interpretation of qualitative, quantitative, and structural information is essential to many aspects of human endeavor, both terrestrial and extra-terrestrial. The maintenance of an improvement in the quality of life throughout the world and the management of resources heavily on the information provided by chemical analysis. Manufacturing industries use analytical data to monitor the quality of raw materials, intermediates and finished products. Progress and research in many areas is dependent on

establishing the chemical composition of man-made or natural materials, and the monitoring of toxic substances in the environment is of ever-increasing importance. Studies of biological and other complex systems are supported by the collection of large amounts of analytical data. Analytical data are required in a wide range of disciplines and situations that include not just chemistry and most other sciences, from biology to zoology, butte arts, such as painting and sculpture, and archaeology. Space exploration and clinical diagnosis are two quite desperate areas in which analytical data is vital. Important areas of application include the following.

**Quality control** (**QC**) in many manufacturing industries, the chemical composition of raw materials, intermediates and finished products needs to be monitored to ensure satisfactory quality and consistency. Virtually all consumer products from automobiles to clothing, pharmaceuticals and foodstuffs, electrical goods, sports equipment, and horticultural products rely, in part, on chemical analysis. The food, pharmaceutical and water industries in particular have stringent requirements backed by legislation for major components and permitted levels of impurities or contaminants. The electronic industry needs analyses at ultra-trace levels (parts per billion) in relation to the manufacture of semi-conductor materials. Automated, computer-controlled procedures for process-stream analysis are employed in some industries.

## MATERIALS AND METHODS

Metadoxine (Pure) Provided by Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK), Acetonitrile for HPLC from Merck.

**HPLC METHOD DEVELOPMENT:**

**TRAILS**

**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. ODS C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

**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 diluent.

**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 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.

**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)

**Procedure:**

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

**PRECISION**

**REPEATABILITY**

**Preparation of Metadoxine Product Solution for Precision:**

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)

Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

The standard solution was injected 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.

**INTERMEDIATE PRECISION:**

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

**Procedure:**

**Analyst 1:**

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

**Analyst 2:**

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

**Accuracy:**

**Procedure:**

Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found, and Amount added for Metadoxine and calculate the individual recovery and mean recovery values.

**ROBUSTNESS:**

The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results.

**For 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)

Take 0.3ml of stock solution in to 10ml of volumetric flask and make up the volume up to mark with diluent.

**Effect of Variation of flow conditions:**

The sample was analyzed at 0.9ml/min and 1.1ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

**Effect of Variation of mobile phase organic composition:**

The sample was analyzed by variation of mobile phase i.e. ACN: Water was taken in the ratio and 60:40, 70:30 instead of 65:35, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

**RESULTS AND DISCUSSION**

**Optimized Chromatogram (Standard)**

| | | |
|---|---|---|
| Column | : | ODS C18 (4.6 x 150mm, 5µm) |
| 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. |

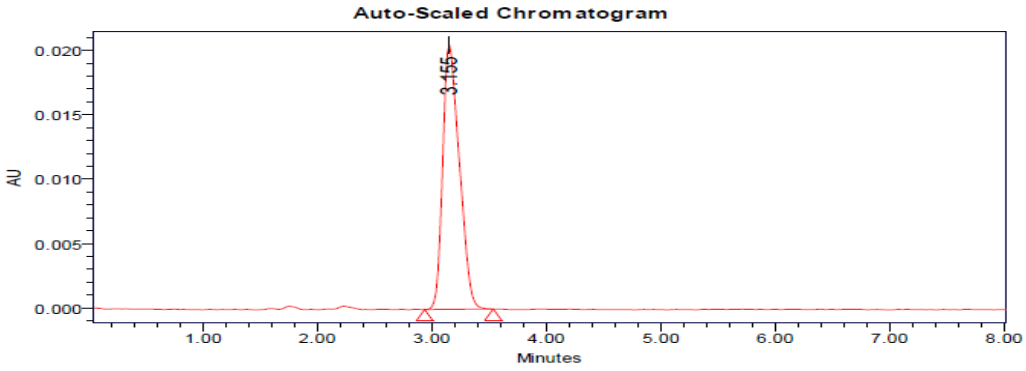


**Figure no.1: Optimized Chromatogram**

**Table no.1: Peak results for Optimized Chromatogram**

| S.No | Peak name | $R_t$ | Area | Height | USP Tailing | USP plate count |
|---|---|---|---|---|---|---|
| 1 | Metadoxine | 3.155 | 206870 | 20497 | 1.30 | 5937 |

**Observation**:

This trial shows proper plate count, peak and baseline in the chromatogram. It's Pass all system suitability parameters. So, it's an optimized chromatogram.

**Optimized Chromatogram (Sample)**

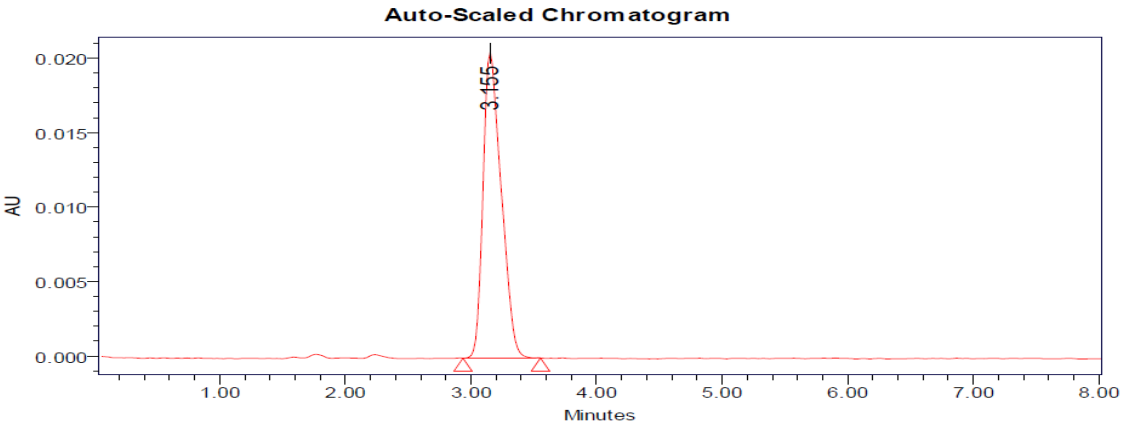


**Figure 2: Optimized Chromatogram (Sample)**

**Table 2: Optimized Chromatogram (Sample)**

| S.No | Name | Retention time(min) | Area (µV sec) | Height (µV) | USP tailing | USP plate count |
|---|---|---|---|---|---|---|
| 1 | Metadoxine | 3.155 | 206198 | 20513 | 1.33 | 5918 |

**SPECIFICITY**

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantitate Metadoxine in drug product.

**Assay (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 | | | |

**Acceptance criteria:**

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

**Assay (Sample):**

**Table 4: Peak results for Assay sample**

| S.No | Name | RT | Area | Height | USP Tailing | USP Plate Count | Injection |
|---|---|---|---|---|---|---|---|
| 1 | Metadoxine | 3.170 | 154627 | 16995 | 1.29 | 5638 | 1 |
| 2 | Metadoxine | 3.174 | 154620 | 16965 | 1.29 | 5602 | 2 |
| 3 | Metadoxine | 3.170 | 153996 | 16754 | 1.26 | 5716 | 3 |

The % purity of Metadoxine in pharmaceutical dosage form was found to be 100.1%.

**LINEARITY**

**Table 5: CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:**

| Concentration Level (%) | Concentration µg/ml | Average Peak Area |
|---|---|---|
| 33 | 10 | 38455 |
| 66 | 20 | 71755 |
| 100 | 30 | 102086 |
| 133 | 40 | 135415 |
| 166 | 50 | 164313 |

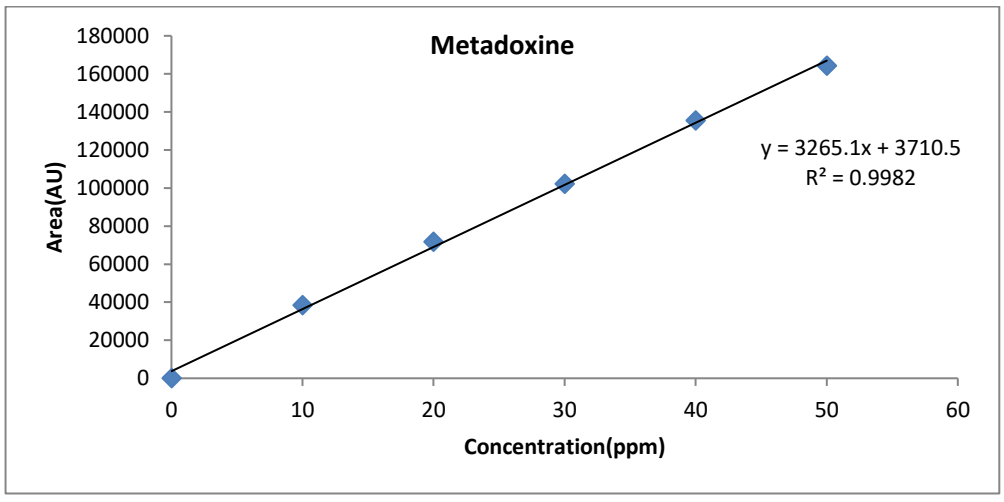


**Figure3: calibration graph for Metadoxine**

**REPEATABILITY**

**Tableno.6: Results of Repeatability 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 | | | |

**Intermediate precision:**

**Table no 7: Results of ruggedness for Metadoxine**

| S.No | PeakName | RT | Area (µV*sec) | Height (µV) | USPPlate count | USPTailing |
|---|---|---|---|---|---|---|
| 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 | | | |

**Table 8: Results of Intermediate precision Analyst 2 for Metadoxine**

| S.No | PeakName | RT | Area (µV*sec) | Height (µV) | USP Plate count | USP Tailing |
|---|---|---|---|---|---|---|
| 1 | Metadoxine | 3.173 | 153634 | 16592 | 5376 | 1.3 |
| 2 | Metadoxine | 3.134 | 153721 | 16538 | 8373 | 1.3 |
| 3 | Metadoxine | 3.161 | 153773 | 16540 | 5827 | 1.3 |
| 4 | Metadoxine | 3.174 | 153957 | 16492 | 5236 | 1.3 |
| 5 | Metadoxine | 3.199 | 153057 | 16593 | 6173 | 1.3 |
| 6 | Metadoxine | 3.199 | 152816 | 16495 | 5927 | 1.3 |
| **Mean** | | | 153493 | | | |
| **Std. Dev.** | | | 450.3301 | | | |
| **% RSD** | | | 0.293388 | | | |

**ACCURACY:**

**Table 9: 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 | |
| 100% | 103318 | 30 | 29.87 | 99.5 | 99.4% |
| 150% | 151061.7 | 45 | 44.79 | 99.5 | |

**Acceptance Criteria:**

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence the method is accurate.

**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 quantitation 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

**Robustness**

**Table 10: Results for Robustness**

| Parameter used for sample analysis | Peak Area | Retention Time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 1.0 mL/min | 126086 | 3.155 | 4245 | 1.33 |
| Less Flow rate of 0.9 mL/min | 139530 | 3.488 | 5372 | 1.3 |
| More Flow rate of 1.1 mL/min | 114279 | 2.877 | 3656 | 1.4 |
| Less organic phase | 116384 | 4.705 | 5362 | 1.4 |
| More organic phase | 113480 | 2.090 | 6251 | 1.2 |

**Acceptance criteria:**

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

**SUMMARY**

**Table 11: Summary of validation data for Metadoxine**

| S.No | Parameter | Observation | Acceptance criteria |
|---|---|---|---|
| 1 | **System suitability** | | |
| | Theoretical plates | 6533 | Not less than 2000 |
| | Tailing | 1.3 | Not more than 2 |
| | %RSD | 0.33526 | Not more than 2.0% |
| 2 | **Specificity** | | |
| | %Assay | 100% | 98-102% |
| 3 | **Method Precision** (%RSD) | 0.02 | Not more than 2.0% |
| 4 | **Linearity** | 10-50 µg/ml | |
| | Slope | 3265 | |

| | | | |
|---|---|---|---|
| | Correlation coefficient($r^2$) | 0.99 | ≤0.99 |
| **5** | **Accuracy** | | |
| | Mean % recovery | 100.00 | 98 - 102% |
| | **Robustness** | All the system | |
| **6** | a) Flow rate variation | suitability | |
| | b) Organic phase variation | parameters are within the limits. | |

## CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Metadoxine in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatization or purification steps.

Metadoxine was freely soluble in ethanol, methanol and sparingly soluble in water.

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 in bulk drug and in Pharmaceutical dosage forms.

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***Corresponding Author:**
***P. Aravinda Reddy and Ramya Sri. S***
*Email:* **surapharmalabs1@gmail.com**