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Research

Validated Rp-Hplc Method For Simultaneous Estimation Of Metadoxine In Bulk And Tablet Dosage Form.

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Abstract

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Metadoxine, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Phenomenex Luna C18 (4.6×150mm, 5µm) column using a mixture of Acetonitrile: Water (90:10%v/v) as the mobile phase at a flow rate of 0.9ml/min, the detection was carried out at 230nm. The retention time of the Metadoxine was 2.826 ±0.02min. The method produce linear responses in the concentration range of 10-50mg/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.

Keywords: Metadoxine, RP-HPLC, validation.

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called “Pharmacopoeia” (e.g. IP, USP, and BP) Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications.

Every year number of drugs are introduced into the market. Also quality is important in every product or service, So, it becomes necessary to develop new analytical methods for such drugs (Dr. Kealey and P.J Haines)

Classification of analytical methods

The following techniques are available for separation and analysis of components of interest.

Spectral methods

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted (or) transmitted etc. E.g.UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry (A.braithwait and F.J.Smith)

Electro analytical methods

Electro analytical methods involved in the measurement of current voltage or resistance as a property of concentration of the component in solution mixture. E.g. Potentiometry, Conductometry, Amperometry.

Chromatographic methods

Chromatography is a qualitative and quantitative analytical technique where in a sample mixture is subjected to preferential separation into different components under the influence of a moving phase (mobile phase) over a stationary phase. These separated components are quantified then by suitable parameter. Chromatography techniques are E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC).

Miscellaneous Techniques

Thermal Analysis DSC, DTA, Gravimetry etc.

Hyphenated Techniques

GC-MS (Gas Chromatography – Mass Spectrometry), LC-MS (Liquid Chromatography – Mass Spectrometry), ICP-MS (Inductivity Coupled Plasma- Mass Spectrometry), GC-IR (Gas Chromatography – Infrared Spectroscopy), MS-MS (Mass Spectrometry – Mass Spectrometry). Analytical techniques that are generally used for drug analysis also include biological and microbiological methods, radioactive methods and physical methods etc (A.braithwait and F.J.Smith)

Introduction to HPLC

HPLC is also called as high pressure liquidchromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

1. Improved resolution of separated substances
2. column packing with very small (3, 5 and 10 μm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Reproducibility
6. continuous flow detectors capable of handling small flow rates
7. Easy sample recovery, handling and maintenance

Types of HPLC Techniques

Based on Modes of Chromatography

These distinctions are based on relative polarities of stationary and mobile phases

Reverse phase chromatography

In this the stationary phase is non-polar and mobile phase is polar. In this technique, the polar compounds are eluted first and non polar compounds are retained in the column and eluted slowly. Therefore it is widely used technique.

Normal phase chromatography

In this the stationary phase is polar and mobile phase is non-polar. In this technique least polar compounds travel faster and are eluted first where as the polar compounds are retained in the column for longer time and eluted**.**

Based on Principle of Separation

Liquid/solid chromatography (Adsorption)

LSC, also called adsorption chromatography, the principle involved in this technique is adsorption of the components onto stationary phase separation of the components occurs by affinity of components toward stationary phase.when the sample solution is dissolved in mobile phase and passed through a column of stationary phase. The basis for separation is the selective adsorption of polar compounds; analytes that are more polar will be attracted more strongly to the active silica gel sites. The solvent strength of the mobile phase determines the rate at which adsorbed analytes are desorbed and elute.It is widely used for separation of isomers and classes of compounds differing in polarity and number of functional groups. It works best with compounds that have relatively low or intermediate polarity.

Liquid/Liquid chromatography (Partition Chromatography)

LLC, also called partition chromatography. In liquid-liquid chromatography two phases are liquids. The two liquids must be immiscible. The stationary liquid is present as thin film on an inert solid support and it is usually the more polar liquid(aqueous phase). Separation accurse due to the difference in partition coefficient of solute between the two liquids.

Ion exchange: In this the components are separated by exchange of ions between an ion exchange resin stationary phase and a mobile electrolyte phase. A cation exchange resin is used for the separation of cations and anion exchange resin is used to separate a mixture of anions (Andrea Weston and Phyllisr).

Size exclusion: In this type, the components of sample are separated according to their molecular sizes by using different gels (polyvinyl acetate gel, agarose gel). ex: separation of proteins, polysaccharides, enzymes and synthetic polymers.

Chiral chromatography: In this type of chromatography, optical isomers are separated by using chiral stationary phase.

Affinity chromatography: In this type, the components are separated by an equilibrium between a macromolecular and a small molecule for which it has a high biological specificity and hence affinity (Andrea Weston and Phyllisr)

Based on elution technique

Isocratic separation: In this technique, the same mobile phase combination is used throughout the process of separation. The same polarity or elution strength is maintained throughout the process.

Gradient separation: In this technique, a mobile phase combination of lower polarity or elution strength is followed by gradually increasing polarity or elution strength (Andrea Weston and Phyllisr).

Based on the scale of operation

Analytical HPLC: Where only analysis of samples are done. Recovery of samples for reusing is normally not done, since the sample used is very low. Ex: µg quantities.

Preparative HPLC: Where the individual fractions of pure compounds can be collected using fraction collector. The collected samples are reused. Ex: separation of few grams of mixtures by HPLC(Yuri Kazakevich and Rosario Lobrutto).

Based on type of analysis

Qualitative analysis: Which is used to identify the compound, detect the presence of impurities to find out the number of components. This is done by using retention time values.

Quantitative analysis: This is done to determine the quantity of individual or several components of mixture. This is done by comparing the peak area of the standard and sample (Andrea Weston and Phyllisr).

Instrumentation of HPLC

The basic liquid chromatography consists of six basic units. The mobile phase supply system, the pump and programmer, the sample valve, the column, the detector and finally a means of presenting and processing the results.

Mobile phase (solvent) reservoirs and solvent degassing

The mobile phase supply system consists of number of reservoirs (200 mL to 1,000 mL in capacity). They are usually constructed of glass or stainless steel materials which are chemically resistant to mobile phase**.**

Mobile phase

Mobile phases in HPLC are usually mixtures of two or more individual solvents. The two most critical parameters for nonionic mobile phases are strength and selectivity.

Mobile phase preparation

Mobile phases must be prepared from high purity solvents, including water that must be highly purified. Mobile phases must be filtered through ≤1 µm pore size filters and be degassed before use.

Degassing of solvents

Many solvents and solvent mixtures (particularly aqueous mixtures) contain significant amounts of dissolved nitrogen and oxygen from the air. These gasses can form bubbles in the chromatographic system that cause both serious detector noise and loss of column efficiency. These dissolved gases in solvent can be removed by the process of degassing. Every solvent must be degassed before introduction into pump as it alter the resolution of column and interfere with monitoring of the column effluent.

Degassing is done in many ways:

1. By warming the solvents
2. By stirring vigorously with a magnetic stirrer
3. By subjecting tovaccum filtration
4. By ultrasonication (using ultrasonicator)
5. By bubbling Helium gas through the solvent reservoir

Pumping systems

The pumping system is one of the most important features of an HPLC system. There is a high resistance to solvent flow due to the narrow columns packed with small particles and high pressures are therefore required to achieve satisfactory flow rate.

There are three basic types of pumps in common use.

1. Reciprocating pumps.
2. Displacement pumps or syringe pumps.
3. Pneumatic pumps or constant pressure pumps.

Sample introduction system

Injection ports are of two basic types,

1. The sample is injected directly into the column.
2. The sample is deposited before the column inlet and then swept by a valving action into the column by the mobile phase.

Injectors should provide the possibility of injecting the liquid sample within the range of 0.1 to 100 mL of volume with high reproducibility and under high pressure (up to the 4000psi).They should also produce minimum band broadening and minimize possible flow disturbances. The most useful and widely used sampling device for modern LC is the micro-sampling injector valve. With these sampling valves, samples can be introduced reproducibly into pressurized columns without significant interruption of flow, even at elevated temperatures. High-performance valves provide extra column band-broadening characteristics comparable or superior to that of syringe injection.

Columns

Typical analytical columns are 10, 15 and 25 cm in length and are fitted with extremely small diameter (3, 5 or 10 µm) particles. The internal diameter of the columns is usually 4 or 4.6 mm; this is considered the best compromise among sample capacity, mobile phase consumption, speed and resolution. Preparative columns are of larger diameter. Packing of the column tubing with the small diameter particles requires high skill and specialized equipment. For this reason, it is generally recommended that the most experienced chromatographers purchase prepacked columns, since it is difficult to match the high performance of professionally packed LC columns without a large investment in time and equipment. The column can be classified based on the material bonded to the silica packed surface such as C_4, C_8, C_{18}, phenyl, chiral, cyanomicrobore columns (1mm to 100cm), U shaped and coiled columns are available. Guard columns are used before the analytical columns to increase the life of analytical columns by retaining non eluted components and particulate matter.

Column Thermostats

Control of column temperature is important in liquid chromatography. The effect of temperature on retention times and reproducibility is quite significant, especially when using the reverse phase models.

Detectors

Optical detectors are most frequently used. These detectors pass a beam of light through the flowing column effluent as it passes through a low volume (~ 10 mL) flow cell. The most commonly used detector in LC is the ultraviolet absorption detector. A variable wavelength detector of this type, capable of monitoring from 190 to 800 nm, will be found suitable for the detection of the majority samples. Other types of Detectors:

1. Refractive index detector
2. Flourimetric detector
3. Conductivity detector
4. Amperometric detector
5. Photodiode array detector (PDA detector).

Data handling

Data handling in chromatography now ranges from a simple pen recorder to complicated computer integration and computerized data handling systems. Several manufacturers today offered microprocessor controlled chromatographs. Thus the solvent delivery system, injector, oven, detector, fraction collector and data reduction can be carried under the control of a central microprocessor with the capability to program sequential parameters.

Applications

HPLC has been used for medical (e.g. detecting vitamin D levels in blood serum), legal (e.g. detecting performance enhancement drugs in urine), research (e.g. separating the components of a complex biological sample, or of similar synthetic chemicals from each other), and manufacturing (e.g. during the production process of pharmaceutical and biological products) purposes.

Chemical Separations It is based on the fact that certain compounds have different migration rates given a particular column and mobile phase, the extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase.

Purification: Purification is defined as the process of separating or extracting the target compound from a mixture of compounds or contaminants. Each compound showed a characteristic peak under certain chromatographic conditions. The migration of the compounds and contaminants through the column need to differ enough so that the pure desired compound can be collected or extracted without incurring any other undesired compound.

Identification Generally assay of compounds are carried using HPLC. The parameters of this assay should be such that a clean peak of the known sample is observed from the chromatograph. The identifying peak should have a reasonable retention time and should be well separated from extraneous peaks at the detection levels which the assay will be performed.

Other applications of HPLC: Other applications of HPLC includes

Pharmaceutical applications

- Tablet dissolution study of pharmaceutical dosages form.
- Shelf-life determinations of pharmaceutical products
- Identification of active ingredients of dosage forms
- Pharmaceutical quality control

Environmental applications

- Detection of phenolic compounds in Drinking Water
- Identification of diphenhydramine in sedimented samples
- Bio-monitoring of pollutant

Forensics

- Quantification of the drug in biological samples.
- Identification of anabolic steroids in serum, urine, sweat, and hair
- Forensic analysis of textile dyes.
- Determination of cocaine and metabolites in blood

Clinical

- Quantification of ions in human urine Analysis of antibiotics in blood plasma.
- Estimation of bilirubin and bilivirdin in blood plasma in case of hepatic disorders.
- Detection of endogenous neuropeptides in extracellular fluids of brain.

Food and Flavor

- Ensuring the quality of soft drink and drinking water.
- Analysis of beer.
- Sugar analysis in fruit juices.
- Analysis of polycyclic compounds in vegetables.
- Trace analysis of military high explosives in agricultural crops

Analytical method validation

Method validation as per ICH can be defined as Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.

Objective of validation

There are two important reasons for validating assays in the pharmaceutical industry.The first, and by for most important is that assay validation is an integral part of the quality control system.The second is that current good manufacturing practice regulation requires assay validation. In industry it would be difficult to confirm that the product being manufactured is uniform and that meet the standards set to assure fitness for use. The varying nature of the differences between the analytical development laboratory and quality control laboratory is a good reason for validation program.

Method validation study includes Specificity / Selectivity, Linearity, Accuracy, Precision, Limit of detection, Limit of Quantitation, Robustness, System suitability and Stability criteria (Breaux J and Jones K).

Accuracy

The accuracy of measurement is defined as the closeness of the measured value to the true value. In a method with high accuracy, a sample (whose “true value” is known) is analyzed and the measured value should ideally be identical to the true value. Typically, accuracy is represented and determined by recovery studies but there are three ways to determine accuracy:

1. Comparison to a reference standard
2. Recovery of the analyte spiked into blank matrix or
3. Standard addition of the analyte.

The ICH documents recommended that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentrations levels the specified range (i.e., three concentrations and three replicates of each concentration). Accuracy was tested (%Recovery and %RSD of individual measurements) by analyzing samples at least in triplicate, at each level (80%, 100% and 120% of label claim) is recommended. For each determination fresh samples were prepared and assay value is calculated.

Precision

Precision can be defined as “the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample”. A more comprehensive definition proposed by the International Conference on Harmonization (ICH) divides precision into three types:

1. Repeatability
2. Intermediate precision
3. Reproducibility

Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision and involves multiple measurements of the same sample (different preparations) by the same analyst under the same conditions. At least

1. 5 or 6 determinations
2. At two or three different concentrations

Intermediate precision: It is the agreement of complete measurements (including standards) when the same method is applied many times within the same laboratory. Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment etc.

Reproducibility: Reproducibility expresses the precision between laboratories and is often determined in collaborative studies or method transfer experiments.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration (amount) of analyte in the sample. It is a measure of how well a calibration plot of response vs. concentration approximates a straight line. Linearity can be assessed by performing single measurements at several analyte concentrations. The data are then processed using a linear least squares regression. The Resulting plot slope, intercept and correlation coefficient provide the desired information on linearity.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present; these include impurities, degradants and matrix etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures. This definition has the following implications:

Identification: To ensure the identity of an analyte.

Purity Tests: To ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content etc.

Assay (content or potency): To provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample (Breaux J and Jones K).

Limit of Detection

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 can be defined as the smallest level of analyte that gives a measurable response. The detection limit is usually expressed as the concentration of the analyte (percentage parts per million) in the sample. It is usually determined by 3 ways:

1. Based on Visual Evaluation
2. Based on Signal-to-Noise
3. Based on the Standard Deviation of the Response and the Slope

Limit of Quantitation

The quantitation limit of an analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOQ is usually expressed as the concentration of the analyte (percentage parts per million) in the sample. . It is usually determined by 3 ways:

1. Based on Visual Evaluation
2. Based on Signal-to-Noise
3. Based on the Standard Deviation of the Response and the Slope

Robustness

It is defined as a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It involves a number of method parameters that are varied within a realistic range and the quantitative influence of the variables is determined.

The typical variations are:

- Influence of variations of pH in a mobile phase.
- Influence of variations in mobile phase composition.
- Different columns (different lots and/or suppliers).
- Temperature.
- Flow rate.

System Suitability Testing

Prior to the analysis of samples each day, the operator must establish that the HPLC system and procedure are capable of providing data of acceptable quality. This is accomplished with system suitability experiments, which can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The parameters that can be used to determine system suitability prior to analysis, includes Plate number (N), Tailing factor, k and/or α, Resolution (Rs) and Relative standard deviation (RSD) of peak height or peak area for repetitive injections. Typically at least two of these criteria are required to demonstrate system suitability for any method (Breaux J and Jones K). Acceptance criteria of validation for HPLC

MATERIALS AND METHODS

Metadoxine (Pure)-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC - 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.

Validation

Preparation of mobile phase

Preparation of mobile phase

Accurately measured 100ml (10%) of HPLC Water and 900ml of Acetonitrile (90%) were mixed and degassed by sonication for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Column : Phenomenex Luna C18 (4.6×150mm, 5µm)
Column temperature : 40ºC
Wavelength : 230nm
Mobile phase ratio : Acetonitrile: Water (90:10%v/v)
Flow rate : 0.9ml/min

Injection volume : 10 µl
Run time : 6min

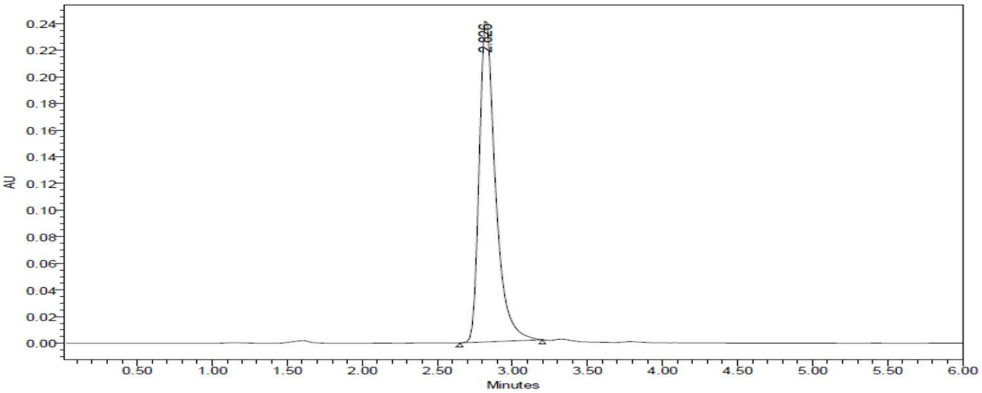


Fig 1: Optimized Chromatogram (Standard)

Table 1: Optimized Chromatogram (Standard)

S.no	Name	RT	Area	Height	USPTailing	USPPlate Count
1	Metadoxine	2.826	1817227	243790	1.4	5200

From the above chromatogram elution of peak is good and it shows proper tailing, platecount in the chromatogram. So it was selected as an optimized chromatogram.

Optimized Chromatogram (Sample)

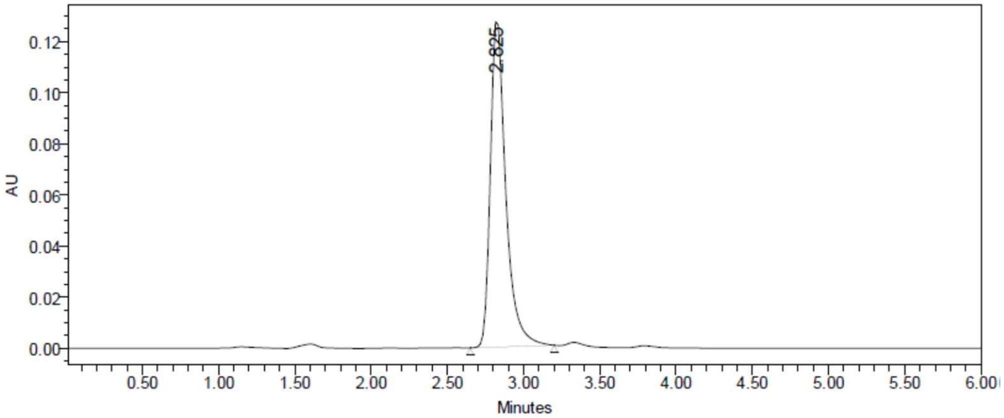


Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USPTailing	USPPlate Count
1	Metadoxine	2.825	898292	128687	1.4	4998

- *Theoretical plates must be not less than 2000*
- *Tailing factor must be not less than 0.9 and not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

System suitability

Table 3: Results of system suitability for Metadoxine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Metadoxine	2.824	1819564	249911	3965	1.4
2	Metadoxine	2.825	1822439	252600	3998	1.4
3	Metadoxine	2.827	1819738	255482	4015	1.4
4	Metadoxine	2.822	1816041	249241	3975	1.4
5	Metadoxine	2.830	1812710	245336	4215	1.4
Mean			1818098			
Std.Dev.			3773.09			
%RSD			0.2			

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

Assay (Standard)

Table 4: Peak results for assay standard

S.No	Name	RT	Area	Height	USPTailing	USPPlateCount	Injection
1	Metadoxine	2.828	892717	124236	1.4	3922.9	1
2	Metadoxine	2.829	899298	124029	1.4	3883.2	2
3	Metadoxine	2.828	891366	125525	1.4	4023.9	3

Assay (Sample)

Table 5: Peak results for Assay sample

S.No	Name	RT	Area	Height	USPTailing	USPPlateCount	Injection
1	Metadoxine	2.826	896838	124869	1.4	3928.6	1
2	Metadoxine	2.825	898292	128687	1.4	4568.8	2
3	Metadoxine	2.833	901496	129200	1.4	5693.0	3

%ASSAY =

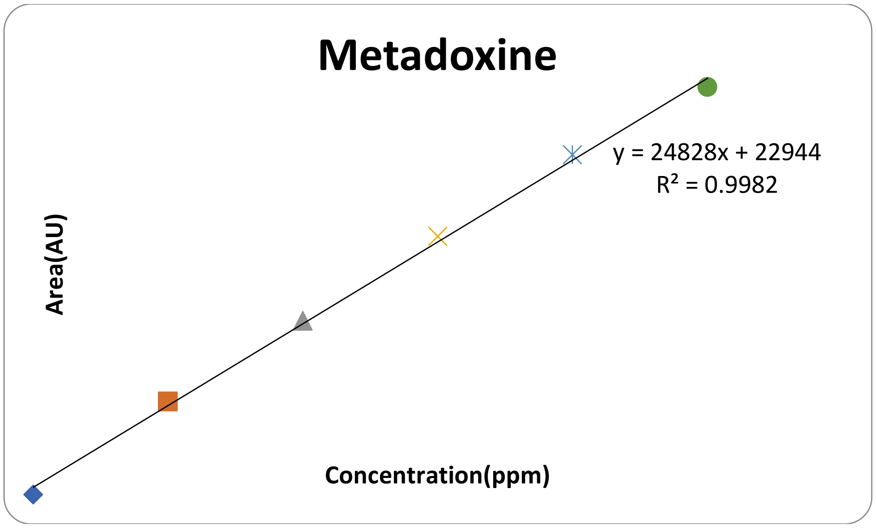
$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Metadoxinein pharmaceutical dosage form was found to be 100.8%.

Linearity

Chromatographic data for linearity study

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	10	282417
66	20	526995
100	30	783937
166	40	1031164
133	50	1237297



Repeatability

Table 6: Results of repeatability for Metadoxine

S. No	Peak name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Metadoxine	2.824	894562	128135	3981	1.3
2	Metadoxine	2.827	896754	129139	4213	1.4
3	Metadoxine	2.833	893627	132891	4562	1.4
4	Metadoxine	2.833	893750	129914	4562	1.4
5	Metadoxine	2.836	892682	130515	4610	1.4
Mean			894275			
Std.dev			1537.936			
%RSD			0.171976			

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Intermediate precision
Day 1

Table 7: Results of Intermediate precision for Metadoxine

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Metadoxine	2.823	895311	125747	4510	1.4
2	Metadoxine	2.827	896783	122578	4002	1.4
3	Metadoxine	2.828	895237	124365	4235	1.4
4	Metadoxine	2.828	894206	124057	4235	1.4
5	Metadoxine	2.825	895085	125410	4015	1.4
6	Metadoxine	2.822	896041	129241	3998	1.3
Mean			895443.8			
Std.Dev.			879.931			
%RSD			0.09			

- *%RSD of Six different sample solutions should not more than 2*

Table 8: Results of Intermediate precision Day 2 for Metadoxine

S.o	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Metadoxine	2.833	893627	132891	4986	1.4
2	Metadoxine	2.836	892682	130515	5123	1.4
3	Metadoxine	2.827	896754	129139	4081	1.4
4	Metadoxine	2.827	896754	129139	4150	1.4
5	Metadoxine	2.823	895311	125747	4051	1.3
6	Metadoxine	2.827	896783	122578	4150	1.4
Mean			895318.5			
Std.Dev.			1793.234			
%RSD			0.2			

- *%RSD of Six different sample solutions should not more than 2*

Accuracy

The accuracy results for Metadoxine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	406872	15	15.0	100.4	100.1%
100%	766853.3	30	29.9	99.8	
150%	1143813	45	45.1	100.3	

- *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 method is accurate.

Robustness

Table 9: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	1817227	2.826	4531	1.4
Less Flow rate of 0.8mL/min	1005760	3.13	4921.2	1.4
More Flow rate of 1.0mL/min	819776	2.589	4493.3	1.4
More Organic phase	922032	2.514	3834.7	1.3
Less Organic phase	893128	3.344	5032.7	1.3

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

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 derivatisation or purification steps. Metadoxine was freely soluble in ethanol, methanol and sparingly soluble in water. Acetonitrile: 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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