



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

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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, 5 μ m) 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 $\pm$ 0.02min respectively. The method produces linear responses in the concentration range of 10-50 μ g/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 chemistry¹

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 ultrasonicator 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	:	35°C
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.

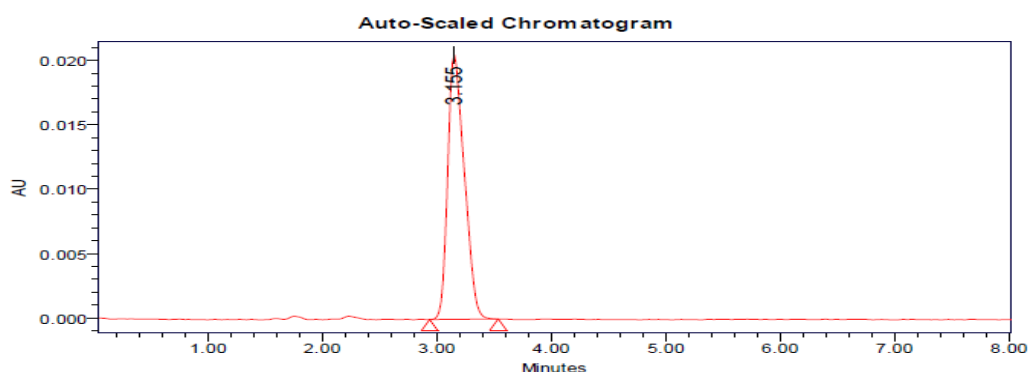


Figure no.1: Optimized Chromatogram

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)

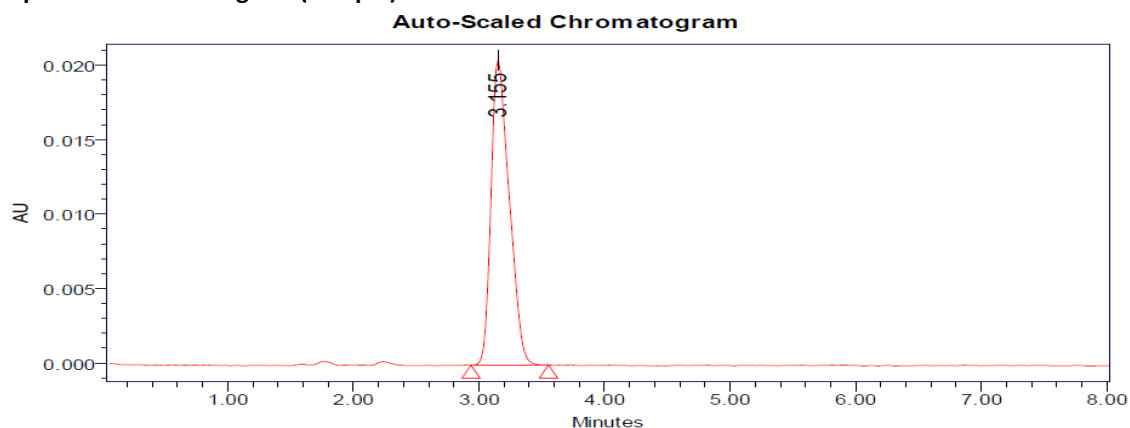


Figure 2: Optimized Chromatogram (Sample)

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 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

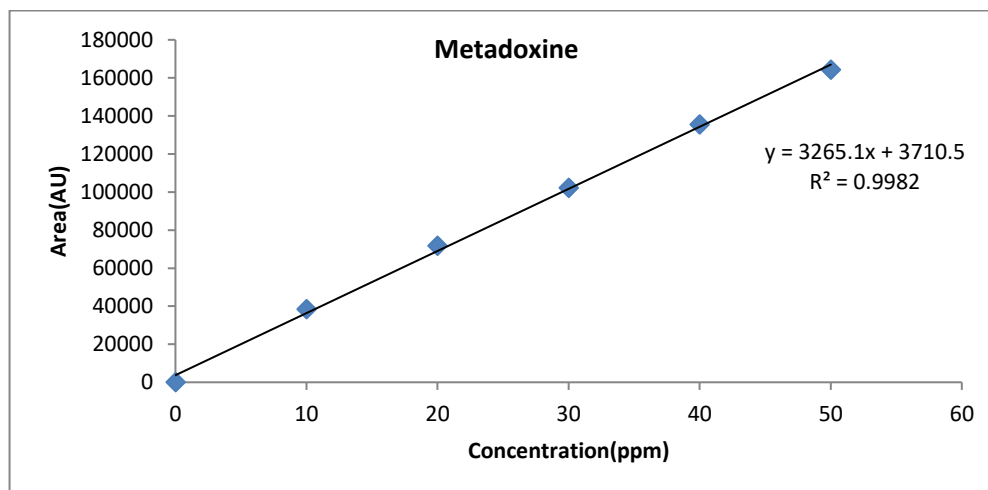


Figure3: calibration graph for Metadoxine

REPEATABILITY

Tableno.6: Results of Repeatability for Metadoxine:

S. No	Peak name	Retention time	Area($\mu\text{V}\cdot\text{sec}$)	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 ($\mu\text{V}\cdot\text{sec}$)	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	3.158	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 ($\mu\text{V}\cdot\text{sec}$)	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	99.4%
100%	103318	30	29.87	99.5	
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.

$$LOD = 3.3 \times \sigma / S$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

$$= 3.3 \times 1314.685 / 3265$$

$$= 1.3 \mu\text{g/ml}$$

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.

$$LOQ = 10 \times \sigma / S$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

$$= 10 \times 1314.685 / 3265$$

$$= 4.0 \mu\text{g/ml}$$

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
2	%RSD	0.33526	Not more than 2.0%
	Specificity		
3	%Assay	100%	98-102%
4	Method Precision (%RSD)	0.02	Not more than 2.0%
4	Linearity	10-50 $\mu\text{g/ml}$	
	Slope	3265	

	Correlation coefficient(r^2)	0.99	≤ 0.99
5	Accuracy		
	Mean % recovery	100.00	98 - 102%
6	Robustness	All the system	
	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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BIBLIOGRAPHY

1. Dr. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, (2002), PP 1-7.
2. A.Braithwait and F.J.Smith, Chromatographic Methods, 5th edition, Kluwer Academic Publisher, (1996), PP 1-2.
3. Andrea Weston and Phyllis.Brown, HPLC Principle and Practice, 1st edition, Academic press, (1997), PP 24-37.
4. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, (2007), PP 15-23.
5. Chromatography, (online).
URL: <http://en.wikipedia.org/wiki/Chromatography>
6. Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, (2004), PP 7-8.
7. Sahajwalla CG a new drug development, vol 141, Marcel Dekker Inc., New York, (2004), PP 421-426.
8. Introduction to Column. (Online), URL: [http://amitpatel745.topcities.com/index_files/study/column care.pdf](http://amitpatel745.topcities.com/index_files/study/column%20care.pdf)
9. Detectors used in HPLC (online)URL: http://wiki.answers.com/Q/What_detectors_are_used_in_HPLC
10. Detectors (online) ,URL: http://hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_uvda.html
11. Detectors (online) ,URL: http://www.dionex.com/enus/w ebdocs/64842-31644-02_PDA-100.pdf
12. Detectors (online), URL: <http://www.ncbi.nlm.nih.gov/pubmed/8867705>
13. Detectors (online), URL: <http://www.chem.agilent.com/Library/applications/59643559.pdf>
14. Detectors (online), URL: <http://hplc.chem.shu.edu/new/hplcbook/detector>
15. Draft ICH Guidelines on Validation of Analytical Procedures Definitions and terminology. Federal Register, vol 60. IFPMA, Switzerland, (1995), PP 1126.
16. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, (1996), PP 1- 8.
17. Introduction to analytical method validation (online), available from:
URL: <http://www.standardbase.hu/tech/HPLC%20validation%20PE.pdf>.
18. Data elements required for assay validation, (online) available from:
URL: <http://www.labcompliance.com/tutorial/methods/default.aspx>.
19. Snyder LR practical HPLC method development, 2nd edition. John Wiley and sons, New York, (1997), PP 180-182.
20. Skoog D A, West D M, Holler FJ: Introduction of analytical chemistry. Sounder college of publishing, Harcourt Brace college publishers. (1994), PP 1-5.
21. Sharma B K, Instrumental method of chemical analysis Meerut. (1999), PP 175-203.
22. Breaux J and Jones K: Understanding and implementing efficient analytical method development and validation.



Journal of Pharmaceutical Technology (2003), 5, PP 110-114.

23. Willard, H. y. Merritt L.L, Dean J.A and Settle F.A "Instrumental methods of analysis" 7th edition CBS publisher and distributors, New Delhi, (1991), PP 436-439.
24. ICH Q2A, "validation of analytical methods, definitions and terminology", ICH Harmonized tripartite guideline, (1999).

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