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"QUANTITATIVE DETERMINATION AND VALIDATION OF CLARITHROMYCIN IN PHARMACEUTICAL USING QUANTITATIVE NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY"

Deepti Aggarwal[1]*, Ram Dayal Gupta[1], Shabnam Ain[2] and Vijay Sharma[3]

[1]Faculty of Oxford College of Pharmacy, Hapur-245301, U.P., India.

[2]Faculty of Sanskar College of Pharmacy and Research, Hapur -201015, U.P., India.

[3]College of Pharmacy, IFTM University, Moradabad-244001, U.P., India.

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***Corresponding Author**
Deepti Aggarwal
Faculty of Oxford College of Pharmacy, Hapur-245301, U.P., India.

1. ABSTRACT

A rapid, specific and accurate proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) method was developed to determine Clarithromycin macrolide antibiotic drug drug in pharmaceutical tablet formulation. The method was based on quantitative NMR spectroscopy (QNMR) using Tetra methyl benzene (TMB) as an internal standard and deuterated dimethylsulfoxide (DMSO-d6) as NMR solvent. For the quantification of the drug, the 1H-NMR signals at 6.87 ppm corresponding to the analyte proton of Clarithromycin drug and internal reference standard (IS) Tetra methyl benzene (TMB) respectively were used. The method was validated for different validation parameters of specificity and selectivity, precision and intermediate precision, linearity, accuracy and robustness. The linearity of the calibration curve for analyte in the desired concentration range was good (R2 =0.9992). The method was accurate and precise. The merit of this method is that no reference standard of analyte drug is required for quantification. The method is non-destructive and can be applied for quantification of Clarithromycin in commercial formulation products. The developed method was validated as per International Conference on Harmonization Guidelines (ICH) with respect to specificity, linearity, accuracy, precision, solution stability and robustness.

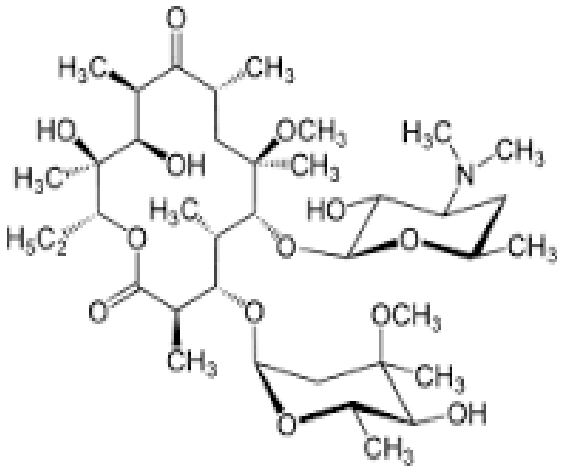
2. KEYWORDS: Clarithromycin, NMR, New Method Development, Validation, QNMR.

3. INTRODUCTION

Clarithromycin is a semisynthetic 14-membered ring macrolide antibiotic used to treat certain bacterial infections such as pneumonia, bronchitis, and infections of the ear, skin and throat. Clarithromycin has the chemical name 6-[4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-14-ethyl-12,13-dihydroxy-4-[5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-7-methoxy-3,5,7,9,11,13-hexamethyl-oxacyclotetradecane-2,10-dione(Figure-1).

Clarithromycin was invented by researchers at the Japanese drug company Taisho Pharmaceutical in 1980. Clarithromycin is an Antibiotic agent, which belongs to the class of medications called macrolide Antibiotics. Clarithromycin, sold under the brand name Crixan, Maclar, synclar and Biaxin are antibiotic used to treat various bacterial infections. This includes strep throat, pneumonia, skin infections, H. pylori infection, and Lyme disease, among others. Clarithromycin can be administered by mouth as a pill or liquid.[1]

Clarithromycin is a bacteriostatic antibiotic used to eradicate H. pylori in the treatment of peptic ulcer disease. The significant role of clarithromycin is one of the wide-spectrum antibiotics used in H. pylori therapy is to prevent protein translation.[2] High Performance Thin Layer Chromatography (HPTLC) has been used to quantify Clarithromycin in many cases. The study reported in the past on Clarithromycin was mainly performed by RPHPLC methods on long columns with higher particle size, which were more time consuming. Even though the method used complex mobile phase mixture with high flow rates, the analysis showed lack of sensitivity and peak symmetry. The purpose of the present study is to develop a simple, sensitive, accurate, precise, rugged and time saving method for the determination of Clarithromycin in formulated product. The target is attained by selecting more advance technique of Waters Acquity UPLC, which gives more accurate result in shorter run time. The method development is performed by optimizing the experimental conditions using mass compatible volatile buffer i.e. Ammonium Acetate, on a shorter column having 1.7µm particle size. The developed method has been validated by following several parameters as mentioned in ICH guideline i.e. linearity, specificity, accuracy, precision, robustness, ruggedness, stability. The pure Active Pharmaceutical Ingredient (API), used in this research work, is manufactured by GLENMARK Company and obtained from Jubilant Generics Limited with a COA (Certificate of Analysis). The Tablet used was MACLAR, manufactured by Macleods Pharmaceutical Ltd.



Chemical formula: $C_{38}H_{69}NO_{13}$

Molecular weight: 747.964 g/mol

Figure 1: Structure of clarithromycin.

4. MATERIAL AND METHOD

4.1 Standard details for nmr

Table 4.1 Standard details.

Standard name	manufacturer	Purity	Storage condition
Clarithromycin	Jubilant generics ltd.	99 %	Stored in a well closed container

4.2 Sample details for nmr

The drug substance used for the validation purpose was manufactured by Glenmark Pharmaceutical LTD.

Table 4.2: Sample details.

Sample name	Batch number	Date of manufacture
Maclar	05141765	JUNE 2015

4.3 Reagents and Solvents

Table 4.3: Solvent details.

S. no	Name	Manufacturer	Grade	Batch no.
1	DMSO d-$_6$	Sigma Aldrich	NMR	LOT#MKBR3576V
2	D_2O	Sigma Aldrich	NMR	LOT#S2BC1895V
3	$CDCl_3$	Sigma Aldrich	NMR	D007H-LOTM0201

4.4 Instrument details

Instruments used for the validation purpose were:

Table 4.4: Instrument details.

S. no.	Instrument	Manufactured by	Instrument ID
1	Brukeravance II(400)	Bruker	JCL/ANAL/NMR/02and03
2	Balance	Mettler Toledo (XS 205 dual range)	JCL/ANAL/BALANCE/03
3	Sonicator	Ultrasonicator	JCL/ANAL/US/01

5. RESULT AND DISCUSSION

5.1 Validation parameters for the assay of clarithromycin by nmr method

Validation is a documented program that provides a high degree of assurance that a facility or operation will consistently produce product meeting a predetermined specifications. According to ICH, it is establishing documented evidence, which provides a high degree assurance that a specific process will consistently produce a product meeting its determined specifications and quality attributes.

The assay procedure was validated for the following parameters.

- Specificity
- Linearity
- Precision
- Intermediate precision
- Accuracy
- Robustness

a) Changing the no. of scans (64±16).

b) Changing the IS (Internal Standard) amount 20% variation (10±2).

5.2 Method validation of clarithromycin

5.2.1 Specificity

Specificity study was performed by analyzing the diluents (DMSO-d_6), placebo solution preparation, Clarithromycin standard preparation, TMB IS preparation and sample (tablet) preparation. It was concluded that there was no interference at the signals obtained at 6.87 ppm for analyte proton and IS respectively due to diluents and placebo. Also the signals of the analyte proton and IS were well separated from each other in standard and sample preparations.

Preparation of sample

Clarithromycin standard preparation for specificity

8.43 mg Clarithromycin standard was weighed accurately and transferred to Stoppard tube and 0.6 ml DMSO-d_6 was added solution was mixed till complete dissolution.

Marketed preparation for specificity

Marketed drug crushed and thoroughly ground in to fine powder. 25.12 mg marketed drug transferred to Stoppard tube. Then 0.6 ml of stock solution of TMB was added. Solution was thoroughly mixed till complete dissolution and supernatant was taken.

Placebo solution preparation for specificity

Accurately weighed 12.50mg of placebo and transferred (mixed of excipient without drug) to Stoppard tube and 0.6 ml of DMSO was added. Solution was thoroughly mixed till the complete dissolution and supernatant was taken for analysis.

TMB (IS) preparation for specificity

10mg TMB in 0.6 ml DMSO- d6 used.

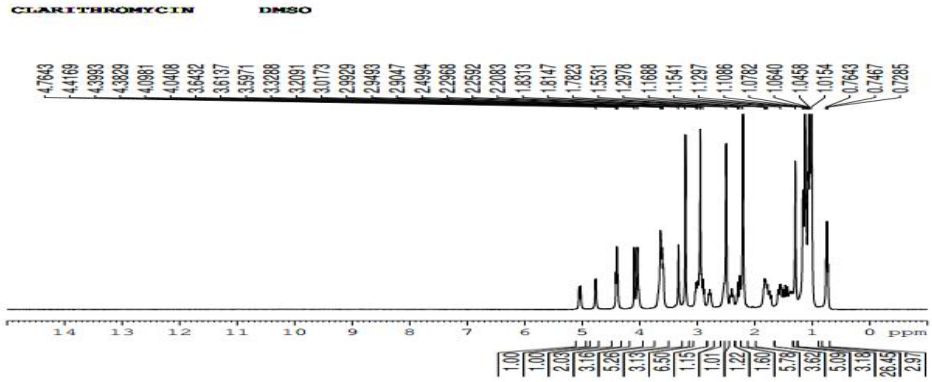


Fig. 5.1: Clarithromycin standard preparation.

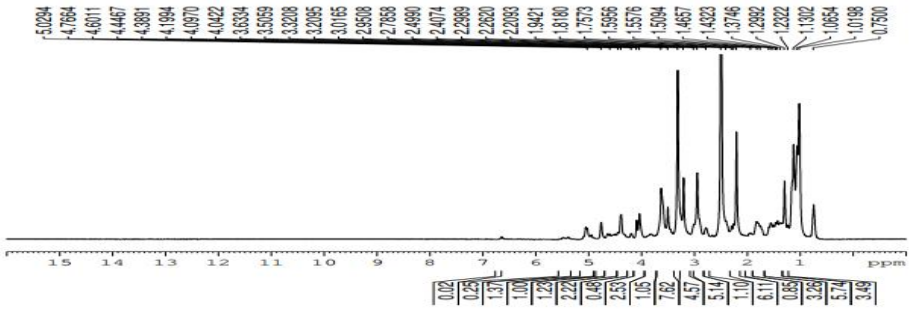


Fig. 5.2: Marketed preparation of clarithromycin.

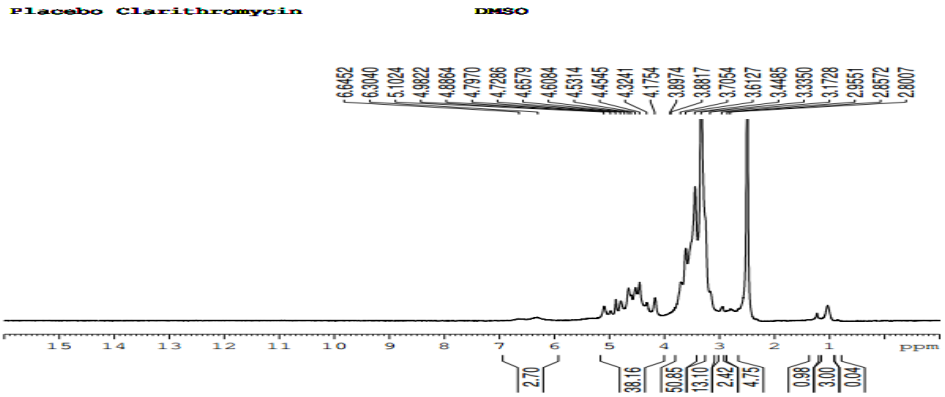


Fig. 5.3: Placebo solution preparation.

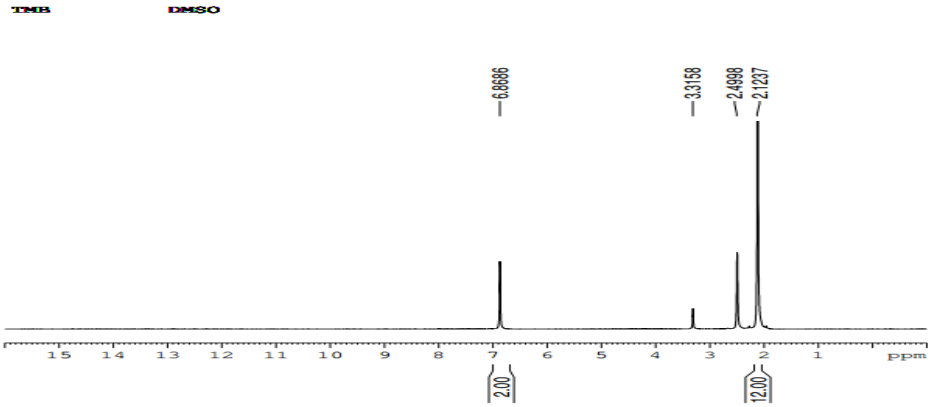


Fig. 5.4: TMB (IS) preparation.

5.2.2 Linearity

Q-NMR as a method itself is liner because the intensity of the response signal is directly proportional to the amount of nuclei contributing to this signal. Linearity was checked by preparing standard solution at triplets of 9 different conc. Levels ranging from 70% to 150%, according to content of analyte in sample. Linearity curve was drawn for taken drug amount (in mg) vs. found drug amount (in mg). The equation for curve was y=1.004x-0.128. The correlation coefficient was found 0.999, indicating good linearity.

Calculation

$$\%\text{Assay} = \frac{I(A) \times M.W(A) \times \text{No. of Hs}(B) \times W(B) \times A(B)}{\text{No. of Hs } (A) \times W(A) \times I(B) \times M.W.(B)}$$

Where,

I (A) = integration value of analyte proton,

M.W. (A) = molecular weight of standard drug,

No. of Hs = no. of highest proton of internal standard (IS),

W (B) = weight of IS,

A (B) = Purity,

No. of Hs (A) = no. of highest proton of standard,

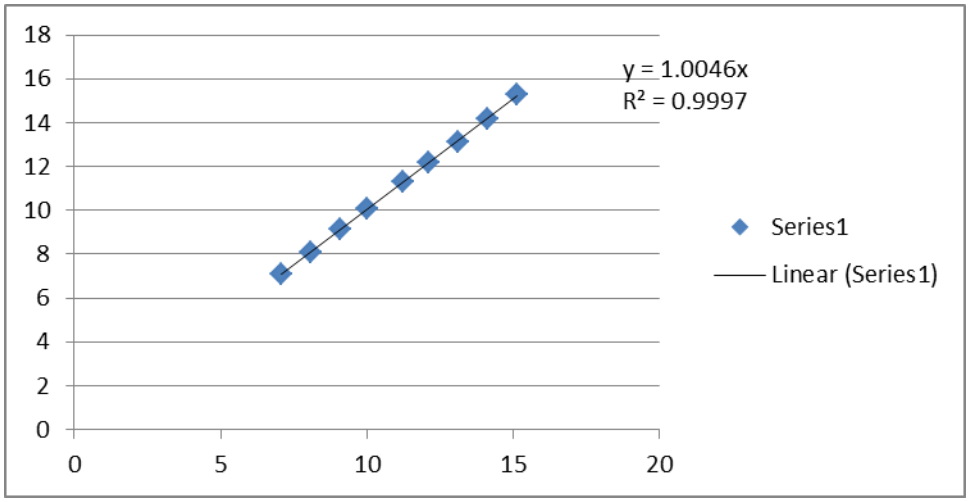
W (A) = weight of standard,

I (B) = integration value of IS,

M.W. (B) = molecular weight of IS.

Table 5.1: Tabular representation of the linearity parameter.

No. of reading	Internal standard (B)*		Compound of interest (A)#		%age	Amount Obtained
	Weight (mg)	Norm. Integration	Weight (mg)	Norm. Integration	Recovery	Weight (mg)
1st	5.00	2.00	7.08	0.26	100.31	7.10
2nd	5.00	2.00	8.07	0.30	100.37	8.10
3rd	5.00	2.00	9.09	0.33	100.13	9.15
4th	5.00	2.00	10.03	0.37	100.76	10.1
5th	5.00	2.00	11.25	0.41	100.29	11.28
6th	5.00	2.00	12.13	0.44	100.54	12.19
7th	5.00	2.00	13.14	0.48	99.77	13.11
8th	5.00	2.00	14.14	0.52	100.39	14.20
9th	5.00	2.00	15.14	0.56	101	15.29



Graph 1: Graphical representation of linearity.

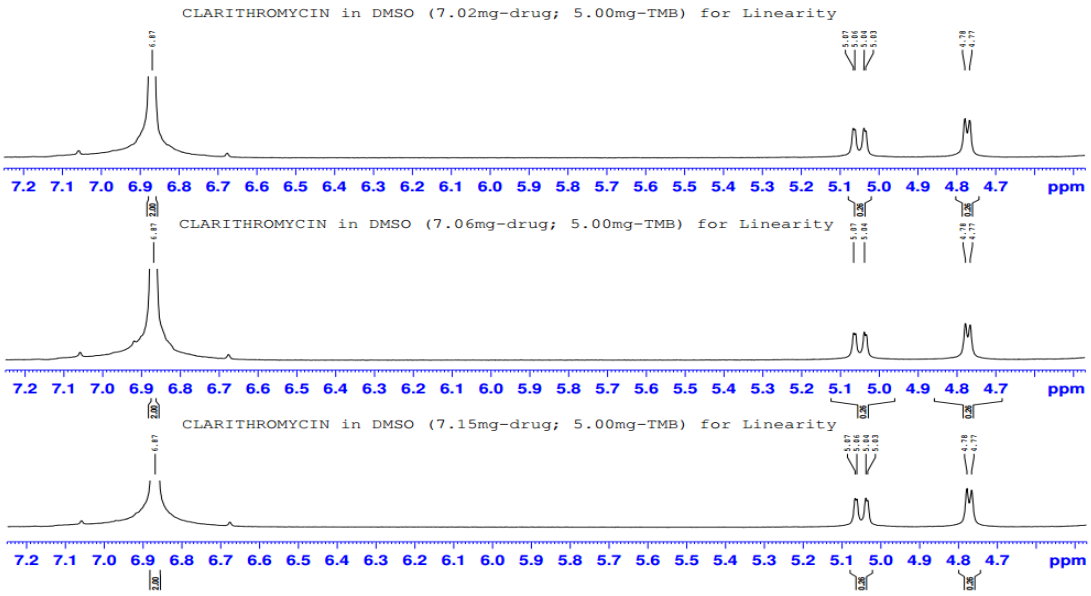


Fig. 5.5.1: Spectra of linearity 7 mg std+ 0.6 ml stock.

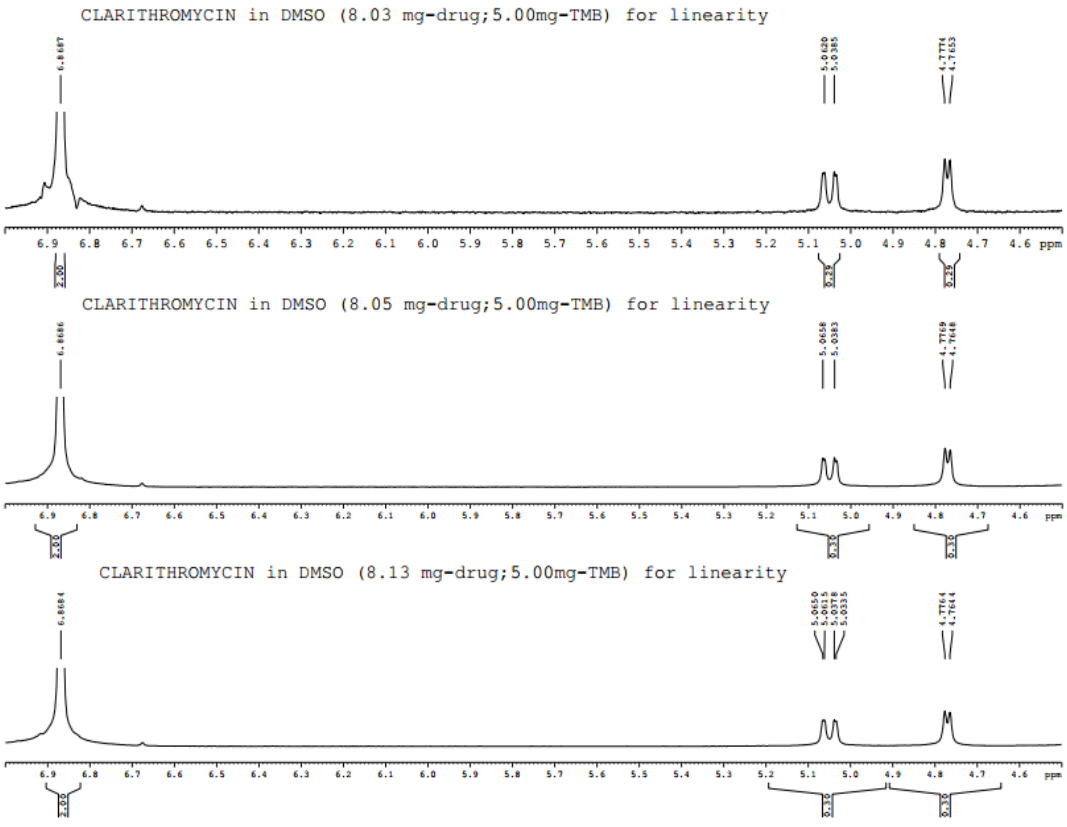


Fig. 5.5.2: Spectra of linearity 8.0 mg std+ 0.6 ml stock.

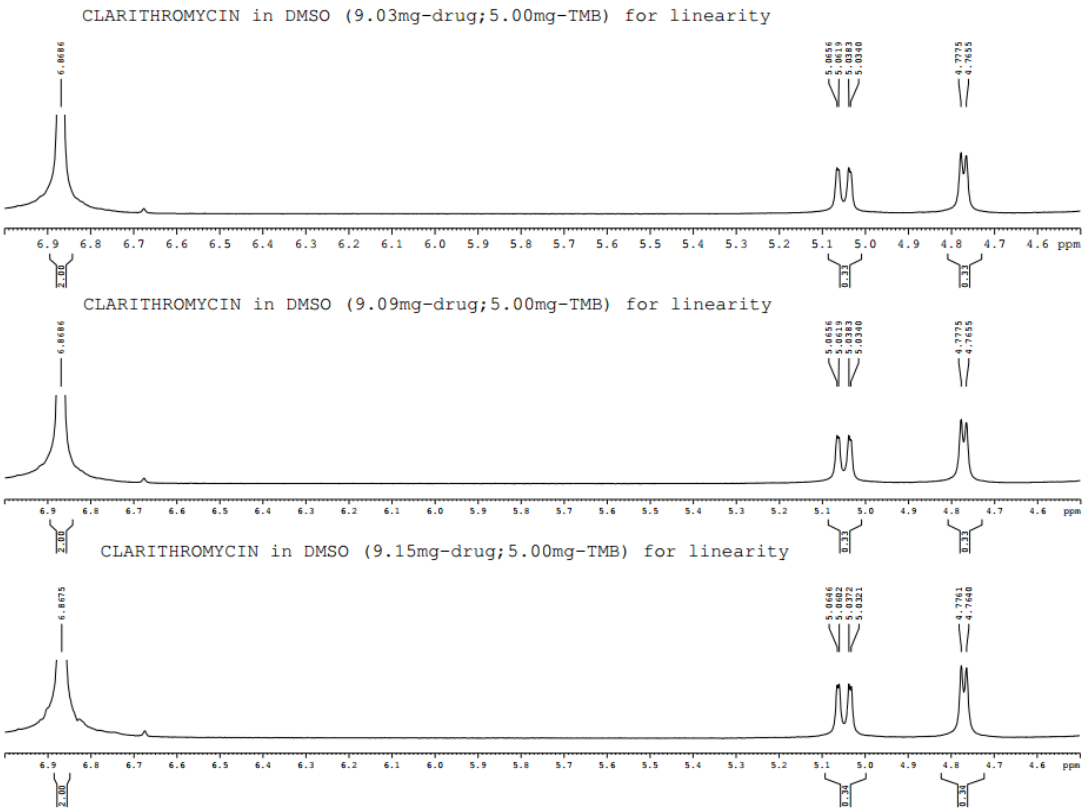


Fig. 5.5.3: Spectra of linearity 9.0 mg std+ 0.6 ml stock.

Fig. 5.5.4: Spectra of linearity 10.0mg std+ 0.6 ml stock.

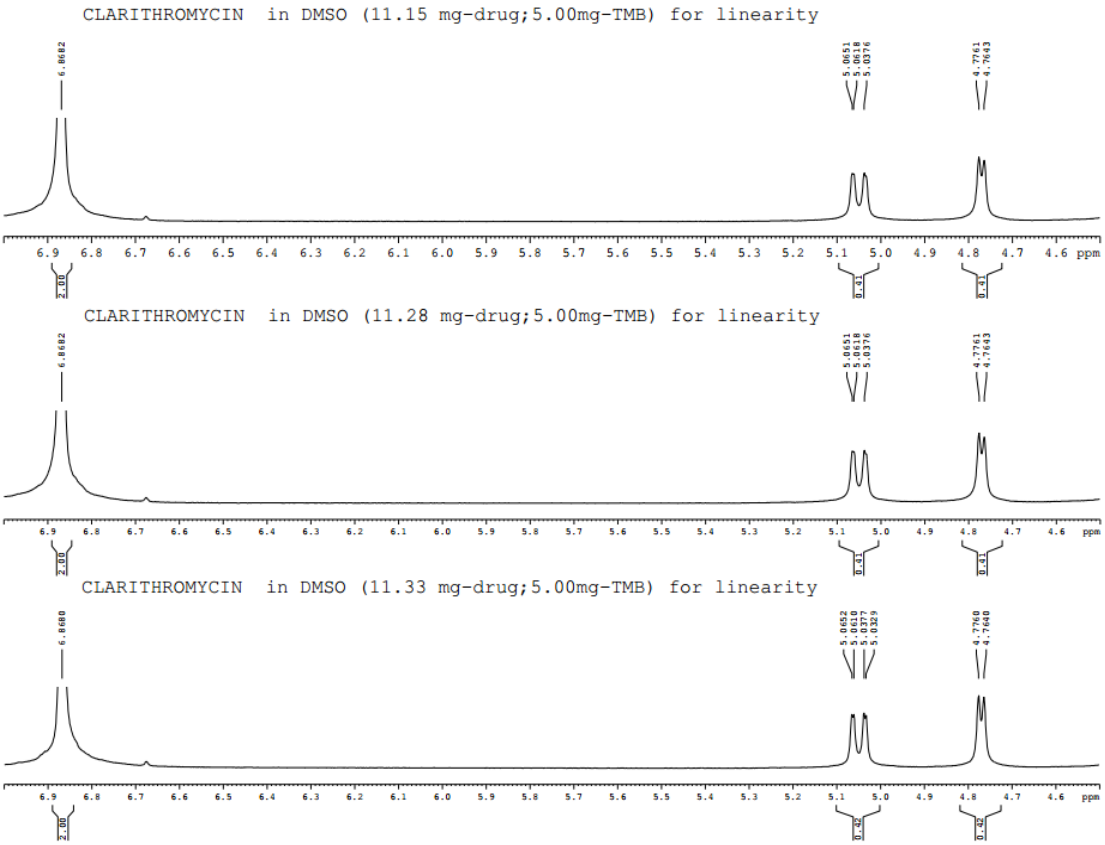


Fig. 5.5.5: Spectra of linearity 11.0 mg std+ 0.6 ml stock.

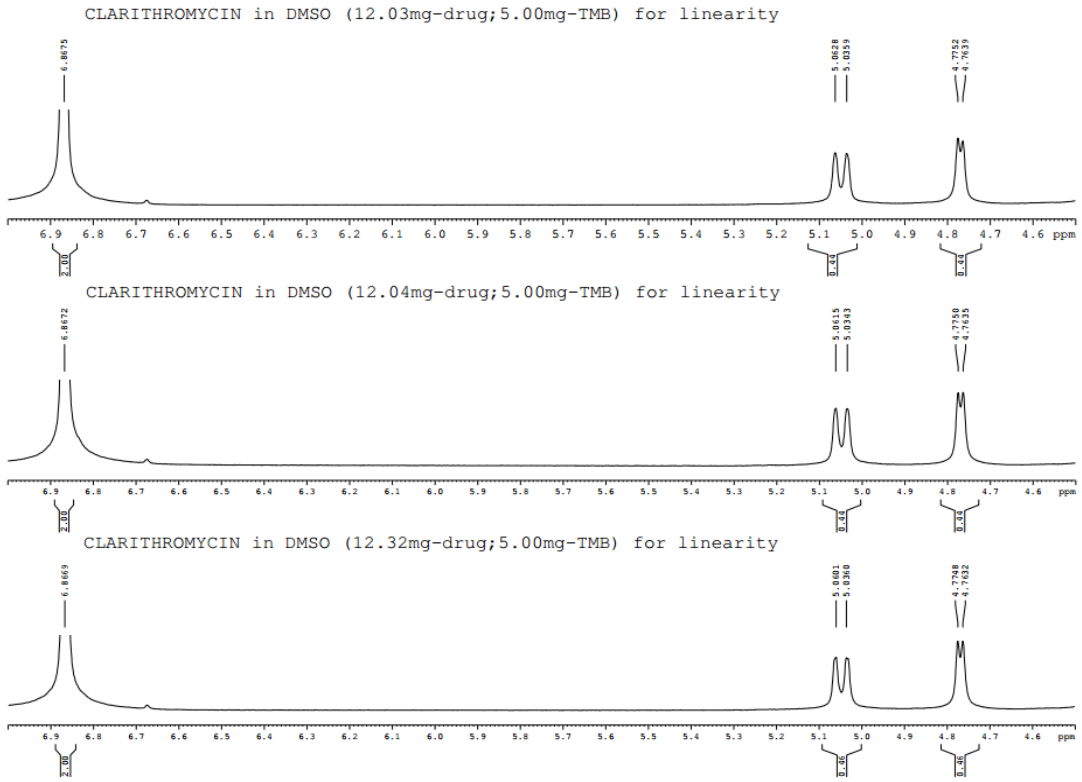


Fig. 5.5.6: Spectra of linearity 12.0 mg std+ 0.6 ml stock.

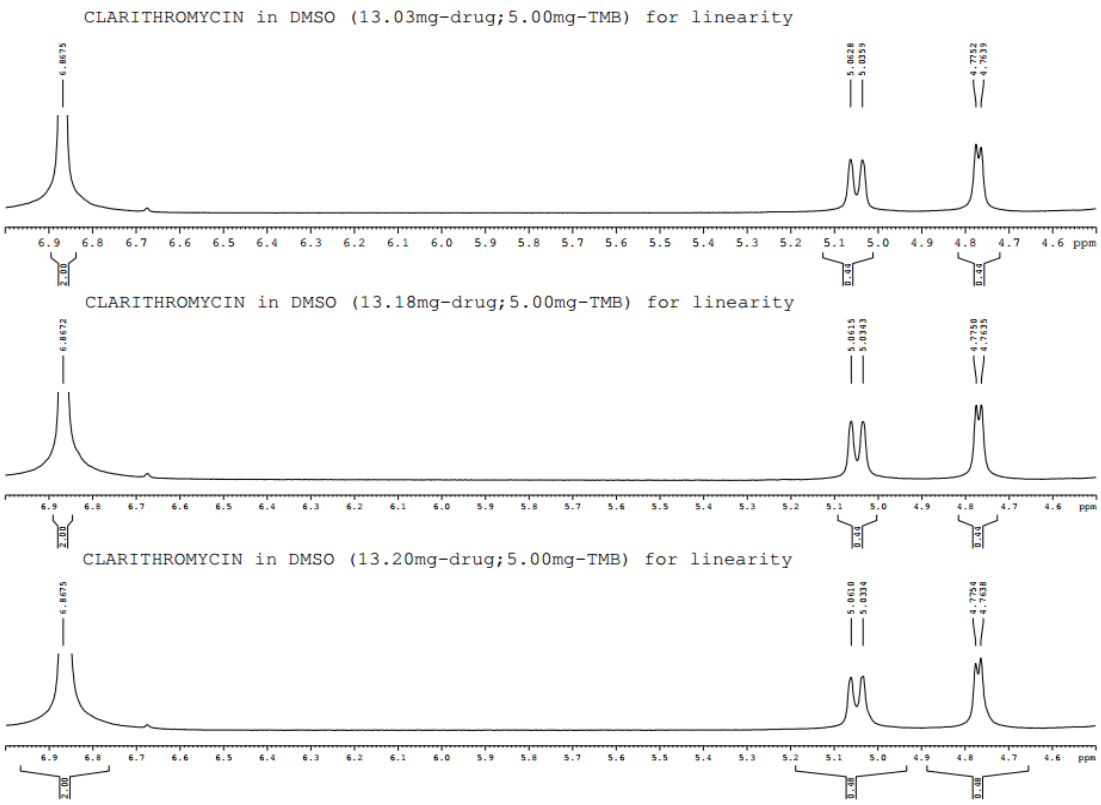


Fig. 5.5.7: Spectra of linearity 13.0 mg std+ 0.6 ml stock.

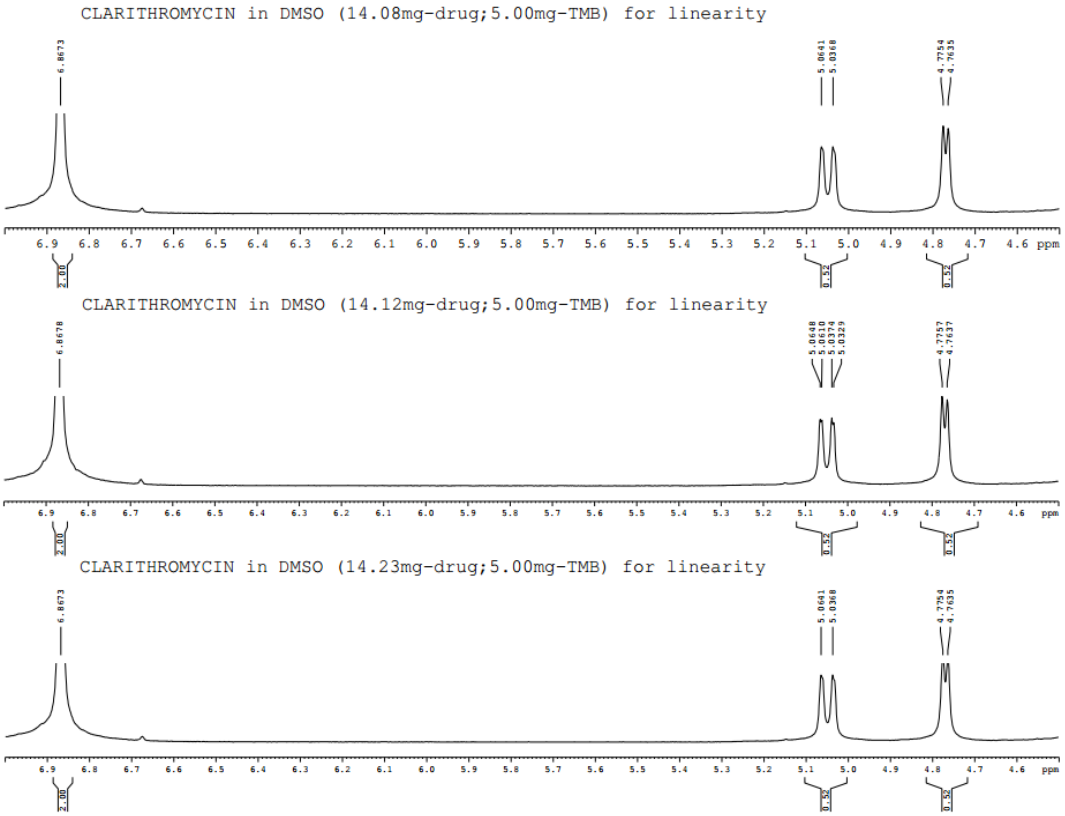


Fig. 5.5.8: Spectra of linearity 14.0 mg std+ 0.6 ml stock.

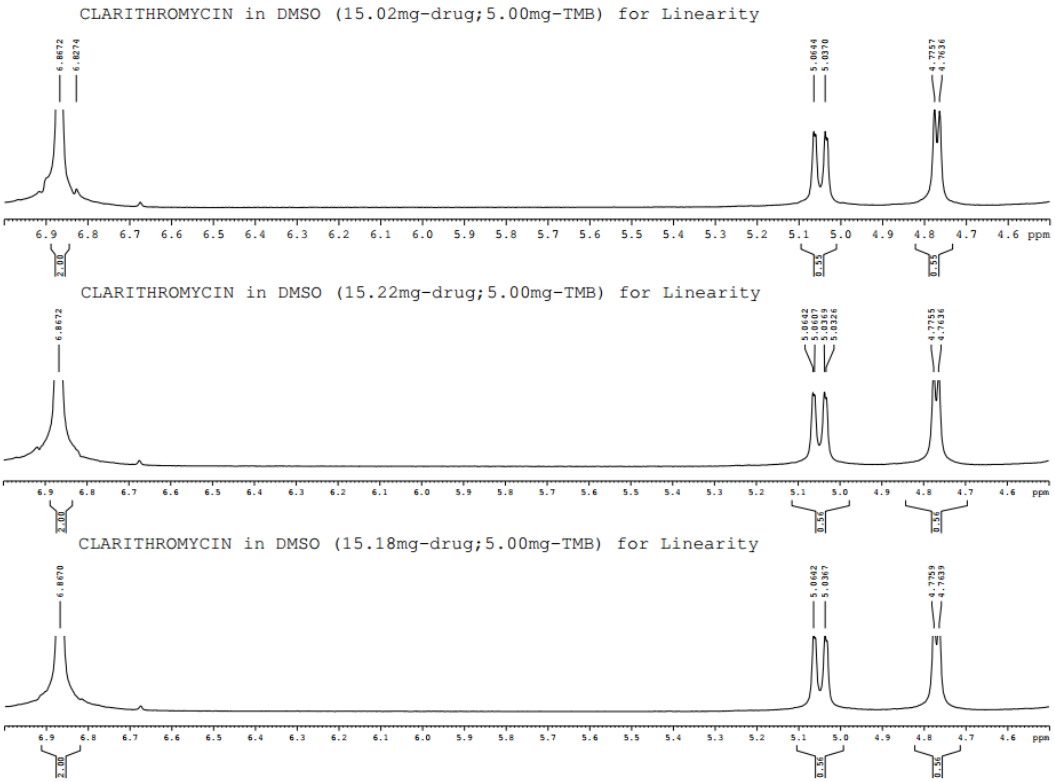


Fig. 5.5.9: Spectra of linearity 15.0 mg std+ 0.6 ml stock.

5.2.3 Precision

The precision of an analytical method expresses the closeness's of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample. According to the ICH guidelines the precision will be acquired by six repeated determinations (n=6). The precision was assessed by six separate sample preparations. Calculated the content of drug in % assay for each preparation and statistical results were tabulated.

Objective: Quantitative analysis of given compound Clarithromycin BY NMR for precision

Experiment: ^{1}H NMR, Solvent DMSO-d_6

Observations

Table 5.2: Tabular representation of the precision parameter.

No. of reading	Internal standard (B)*		Compound of interest (A)#		%age	Amount obtained
	Weight (mg)	Norm. Integration	Weight (mg)	Norm. Integration	Recovery	Weight (mg)
1st	5.00	2.00	10.05	0.37	100.53	10.1032
2nd	5.00	2.00	10.01	0.37	100.93	9.82
3rd	5.00	2.00	10.13	0.37	99.73	10.1026
4th	5.00	2.00	10.11	0.37	99.93	10.1029
5th	5.00	2.00	10.09	0.37	100.13	10.1031
6th	5.00	2.00	10.02	0.37	100.83	10.1031

Constant: - 2730.56

No. of Hs (I.S) =2

No. of Hs (COI) = 1

* I.S. is 1, 2, 4, 5- tetramethylbenzene, M.W. = 134.22, Purity (A (B))= 98%

COI is **clarithromycin,** M.W. =747.95

Formula used

$$\%\text{Assay} = \frac{I(A) \times M.W(A) \times No.\text{of Hs}(B) \times W(B) \times A(B)}{No.\text{of Hs}(A) \times W(A) \times I(B) \times M.W(B)}$$

Table 5.2.1: Precision.

Taken drug in mg	Found drug in mg	%Assay(As such)
10.05	10.1032	100.53
10.01	9.82	100.93
10.13	10.1026	99.73
10.11	10.1029	99.93
10.09	10.1031	100.13
10.02	10.1031	100.83
	Mean	100.35
	SD	0.45
	%RSD	0.45

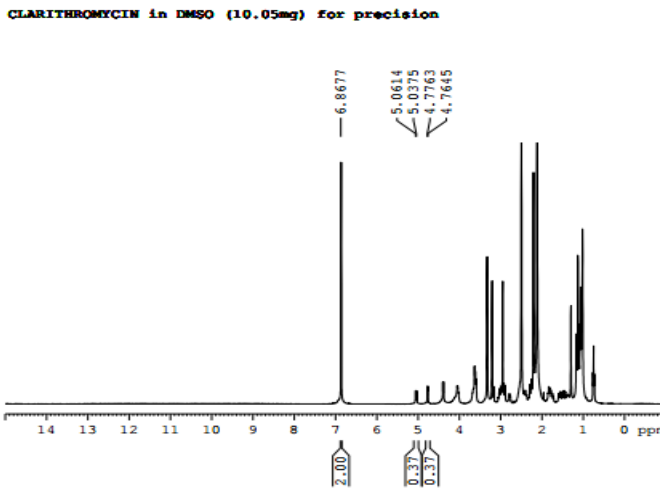


Fig. 5.6.1: Precision 10.05 mg Std + 0.6 ml stock.

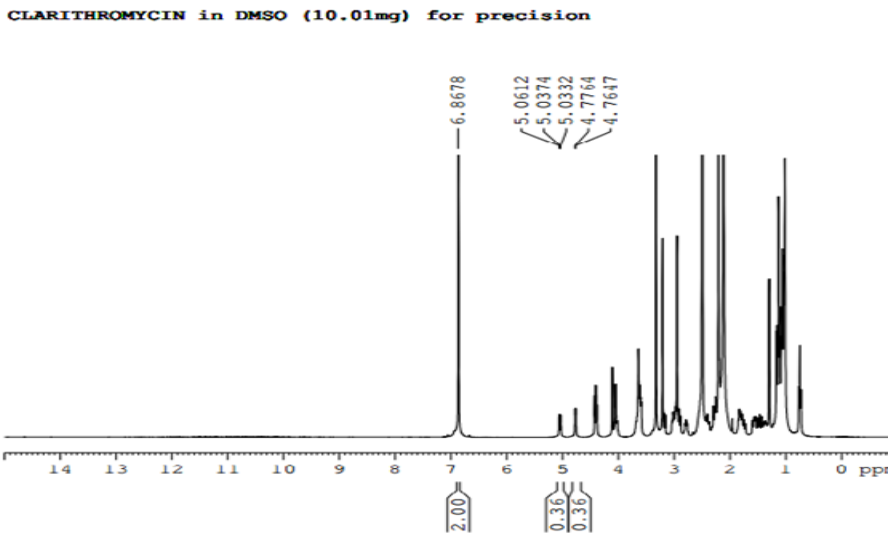


Fig. 5.6.2: Precision 10.01 mg Std + 0.6 ml stock.

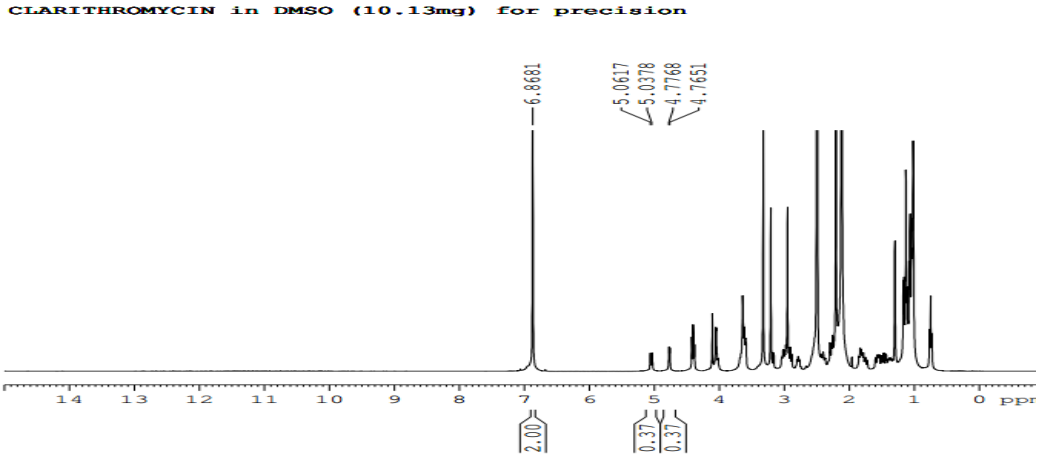


Fig. 5.6.3: Precision 10.13 mg Std + ml stock.

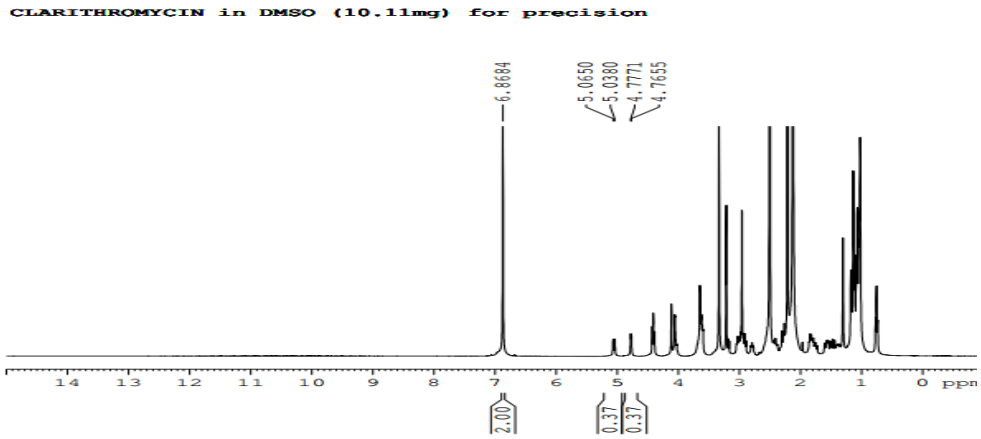


Fig. 5.6.4: Precision 10.11 mg Std + 0.6 ml stock.

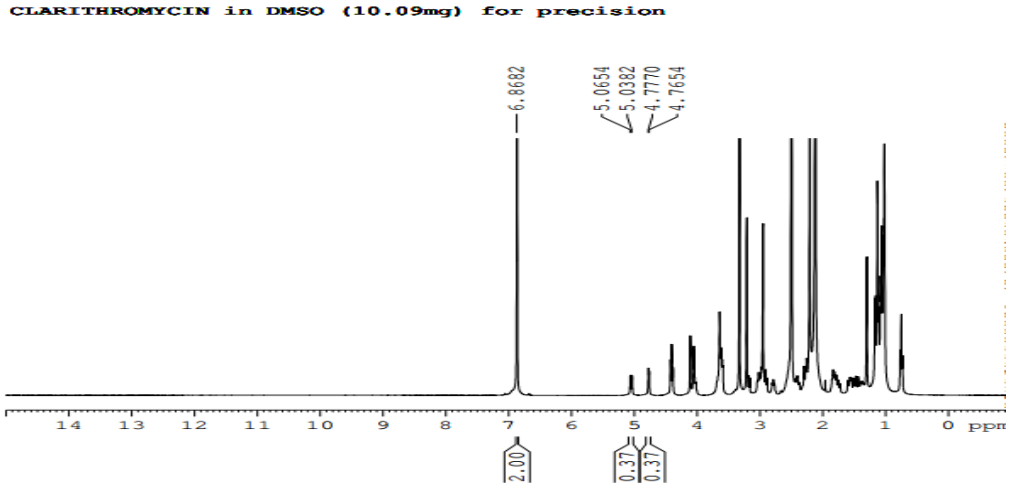


Fig. 5.6.5: Precision 10.09 mg Std + 0.6 ml stock.

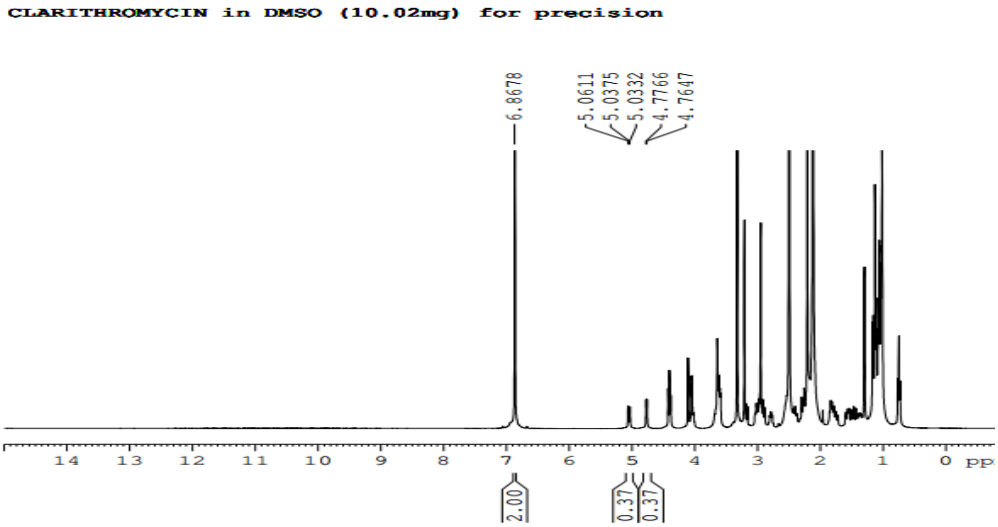


Fig. 5.6.6: Precision 10.02 mg Std + 0.6 ml stock.

5.2.4 Intermediate precision

The intermediate precision will be evaluated by a different analyst on different day and/or different NMR probe and/or a different NMR spectrometer with a different magnetic field strength. Integration of peaks as well as phase and baseline correction is the most subjective parts of the method. Six different sample preparations were prepared and analyzed on different day. The average of six analyses, standard deviation (SD) and RSD values are documented in table.

Objective: Quantitative analysis of given compound Clarithromycin by NMR for Intermediate precision

Experiment: ^{1}H NMR, Solvent DMSO-d_6

Observations

Table 5.3: Tabular representation of the intermediate precision parameter.

No. of reading	Internal standard (B)*		Compound of interest (A)#		%age	Amount obtained
	Weight (mg)	Norm. Integration	Weight (mg)	Norm. Integration	Recovery	Weight (mg)
1st	5.00	2.00	10.07	0.37	100.33	10.1032
2nd	5.00	2.00	10.12	0.37	99.83	10.1027
3rd	5.00	2.00	10.05	0.37	100.53	10.1032
4th	5.00	2.00	10.09	0.37	100.13	10.1031
5th	5.00	2.00	10.02	0.36	98.10	9.8296
6th	5.00	2.00	10.08	0.37	100.23	10.1031

Constant: - 2730.56

No. of Hs (I.S) =2

No. of Hs (COI) = 1

* I.S. is 1, 2, 4, 5- tetramethylbenzene, M.W. = 134.22, Purity (A (B)) = 98%

COI is Clarithromycin**,** M.W. =747.95

Formula used

$$\%\text{Assay} = \frac{I(A) \times M.W(A) \times No.\text{of Hs}(B) \times W(B) \times A(B)}{No.\text{of Hs}(A) \times W(A) \times I(B) \times M.W(B)}$$

Table 5.3.1: Intermediate precision.

Taken drug in mg	Found drug in mg	%Assay(As such)
10.07	10.1032	100.33
10.12	10.1027	99.83
10.05	10.1032	100.53
10.09	10.1031	100.13
10.02	9.8296	98.1
10.08	10.1031	100.23
	Mean	99.86
	SD	0.8142
	%RSD	0.82

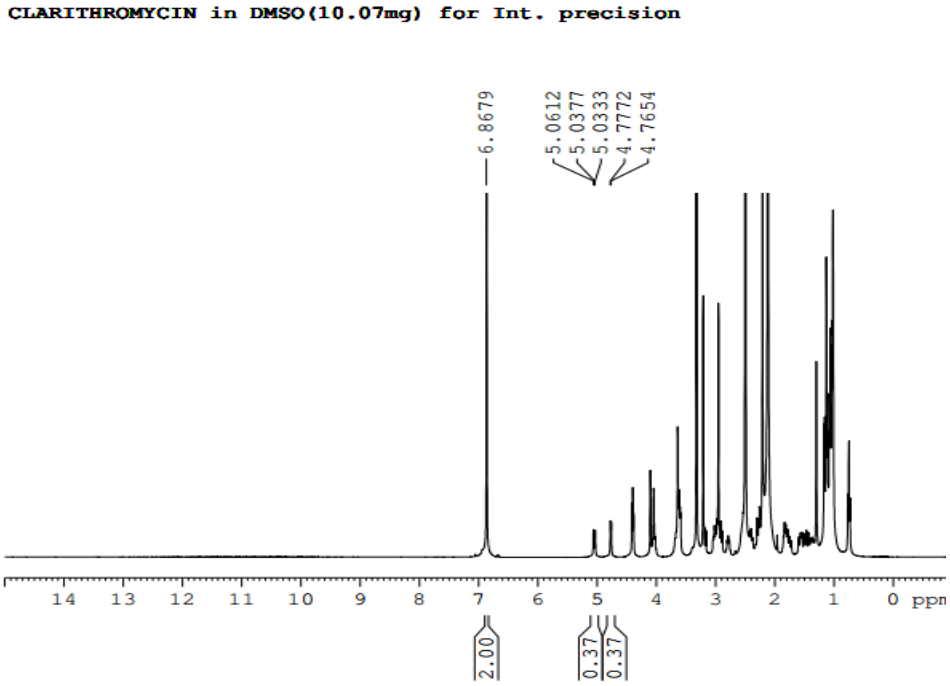


Fig 5.7.1: Intermediate precision 10.07 mg + 0.6 ml stock.

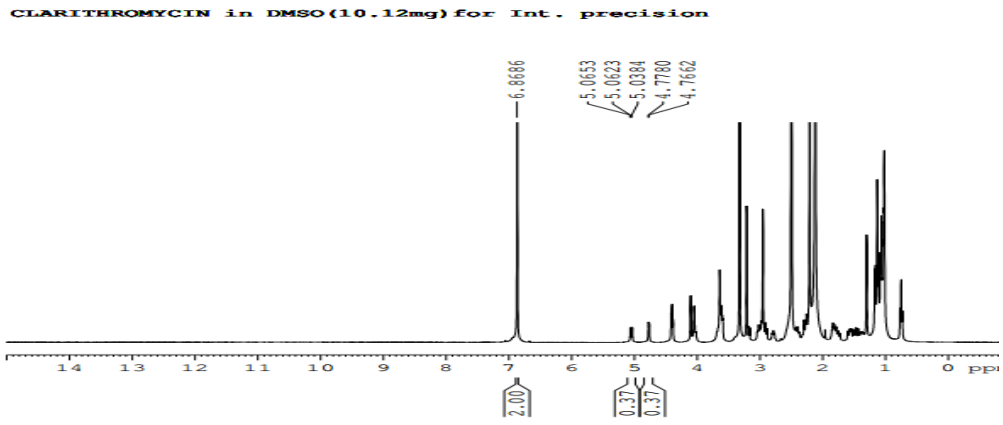


Fig. 5.7.2: Intermediate precision 10.12 mg + 0.6 ml stock.

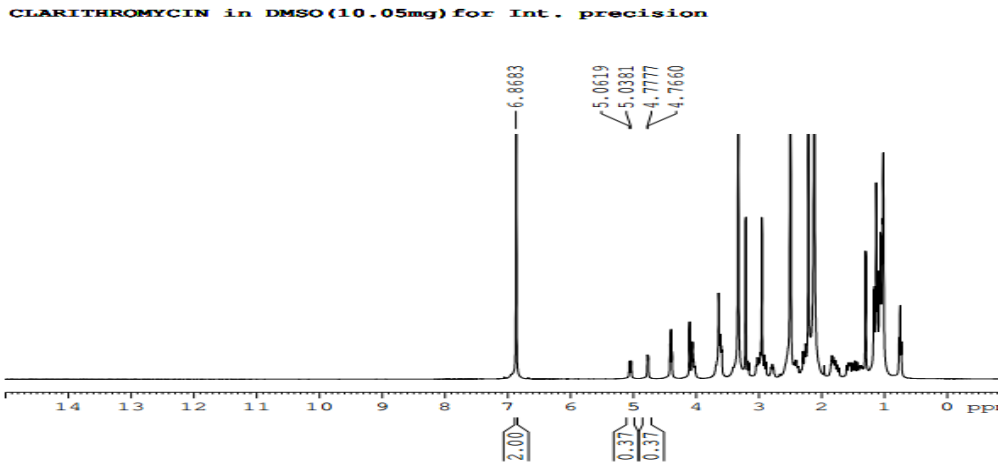


Fig. 5.7.3: Intermediate precision 10.05 mg + 0.6 ml stock.

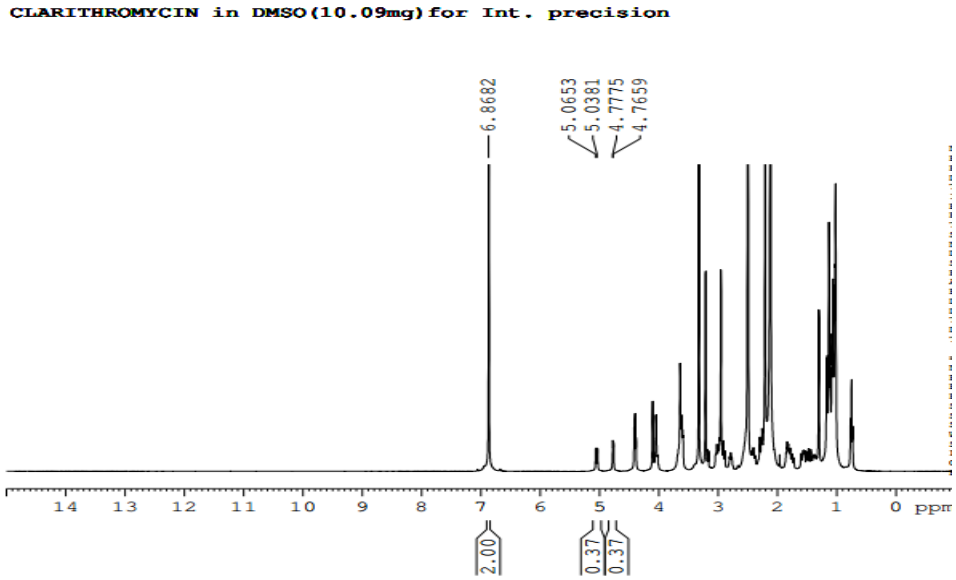


Fig. 5.7.4: Intermediate precision 10.09 mg + 0.6 ml stock.

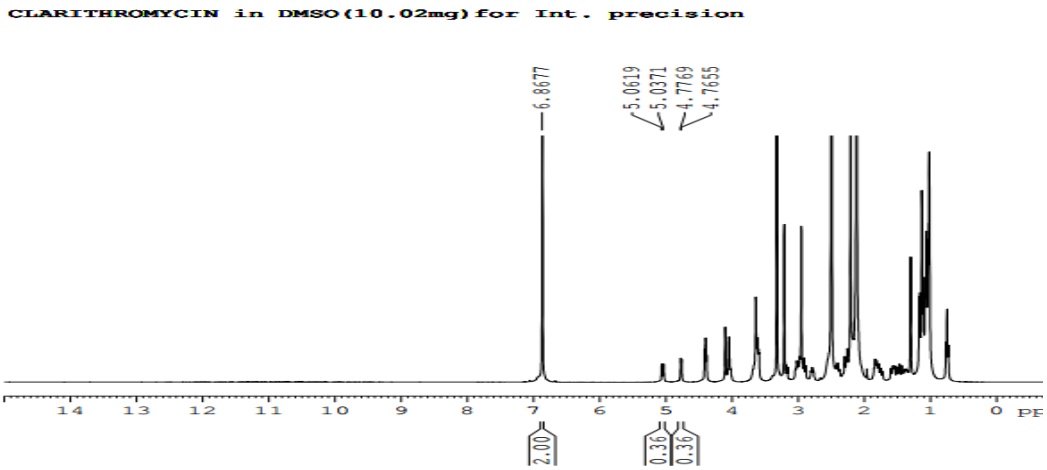


Fig. 5.7.5: Intermediate precision 10.02 mg + 0.6 ml stock.

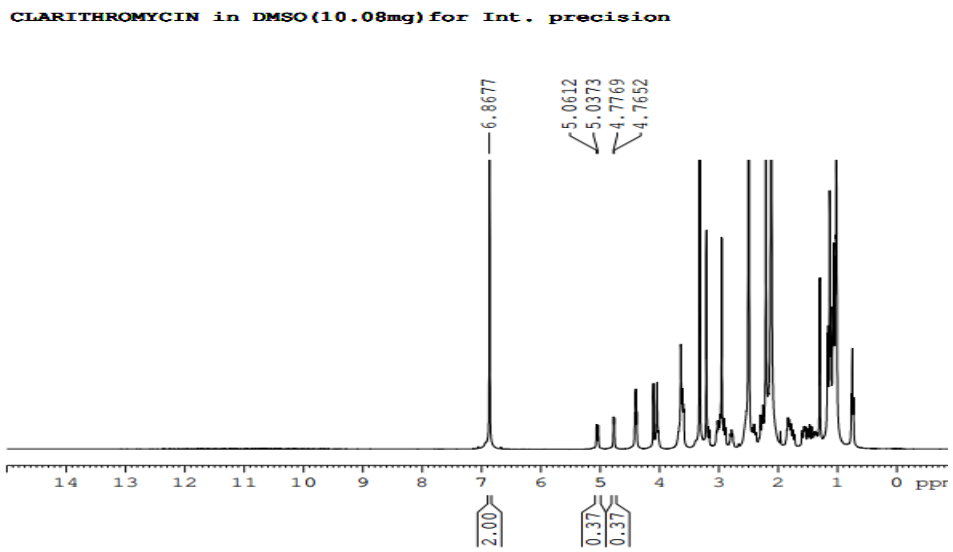


Fig. 5.7.6: Intermediate precision 10.08 mg + 0.6 ml stock.

5.2.5 Accuracy

The accuracy of an analytical method expresses the closeness of agreement between an accepted reference value and the value found. The accuracy of an analytical procedure should be established across its range. The ICH documents recommend that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range.

Data from nine determinations over three concentration levels covering the specified range was determined. The accuracy was studied at 80,100 and 120% levels with respect to the sample by preparing the solutions in triplicate at each level. From the results as per Table, it was concluded that method for assay content was accurate between the ranges of 80 to 120% level.% RSD at each level was level was found to be less than 2.00.

Objective: Quantitative analysis of given compound Clarithromycin by NMR for accuracy

Experiment: ^{1}H NMR, Solvent DMSO-d_6

Observations

Table 5.4: Tabular representation of the accuracy parameter.

No. of reading	Internal standard (B)*		Compound of interest (A)#		%age	Amount obtained
	Weight (mg)	Norm. Integration	Weight (mg)	Norm. Integration	Recovery	Weight (mg)
1st	5.00	2.00	8.03	0.29	98.61	7.92
2nd	5.00	2.00	8.05	0.29	98.37	7.92
3rd	5.00	2.00	8.06	0.29	98.25	7.92
4th	5.00	2.00	10.00	0.36	98.30	9.83
5th	5.00	2.00	10.02	0.36	98.10	9.83
6th	5.00	2.00	10.09	0.36	97.42	9.83
7th	5.00	2.00	12.03	0.44	99.87	12.01
8th	5.00	2.00	12.07	0.44	99.54	12.01
9th	5.00	2.00	12.11	0.44	99.21	12.01

* I.S. is 1,2,4,5- tetramethylbenzene, M.W. = 134.22, Purity (A (B))= 98%

COI is Clarithromycin**,** M.W. =747.95

Formula used

$$\%\text{Assay} = \frac{I(A) \times M.W(A) \times \text{No .of Hs}(B) \times W(B) \times A(B)}{\text{No .of Hs}(A) \times W(A) \times I(B) \times M.W(B)}$$

Observations

Table 5.4.1: Accuracy.

Accuracy level		Taken drug in mg	Found drug in mg	%Assay (as such)
80%	Set-1	8.03	7.92	98.61
80%	Set-2	8.05	7.92	98.37
80%	Set-3	8.06	7.92	98.25
100%	Set-1	10.00	9.83	98.30
100%	Set-2	10.02	9.83	98.10
100%	Set-3	10.09	9.83	97.42
120%	Set-1	12.03	12.01	99.87
120%	Set-2	12.07	12.01	99.54
120%	Set-3	12.11	12.01	99.21
			Mean	98.63
		Overall	SD	0.73
			%RSD	0.74

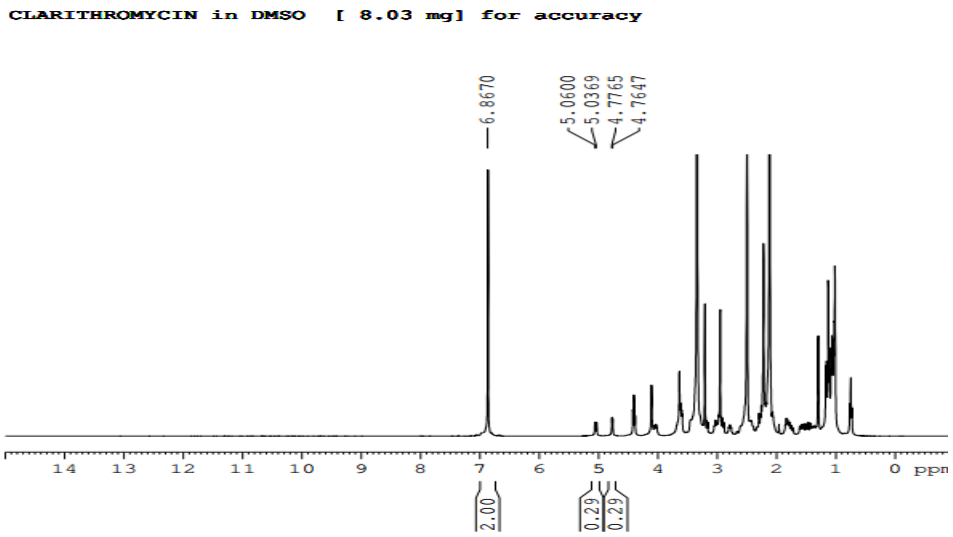


Fig. 5.8.1: Accuracy 8.03 mg Std + 0.6 ml stock.

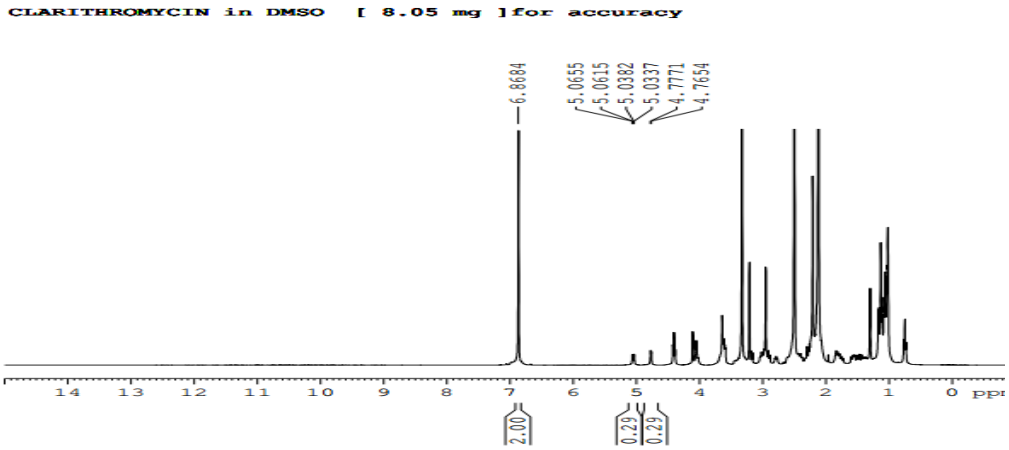


Fig. 5.8.2: Accuracy 8.05 mg Std + 0.6 ml stock.

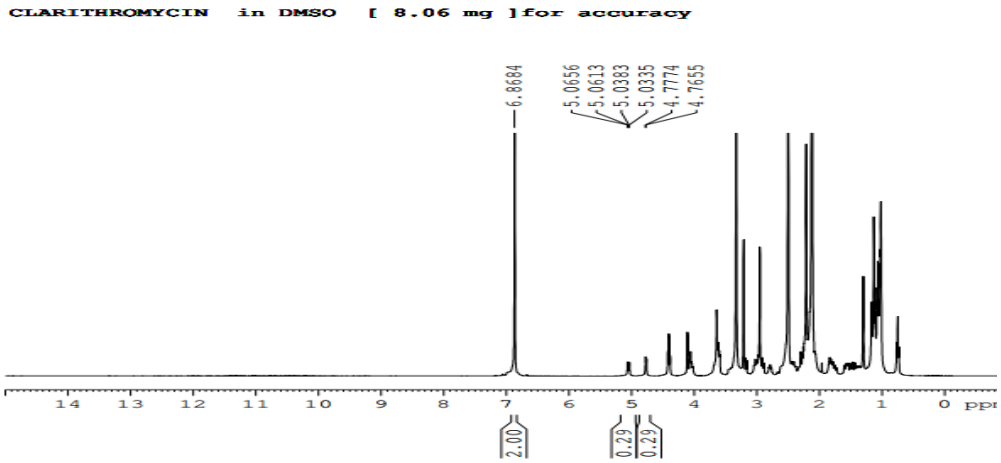


Fig. 5.8.3: Accuracy 8.06 mg Std + 0.6 ml stock.

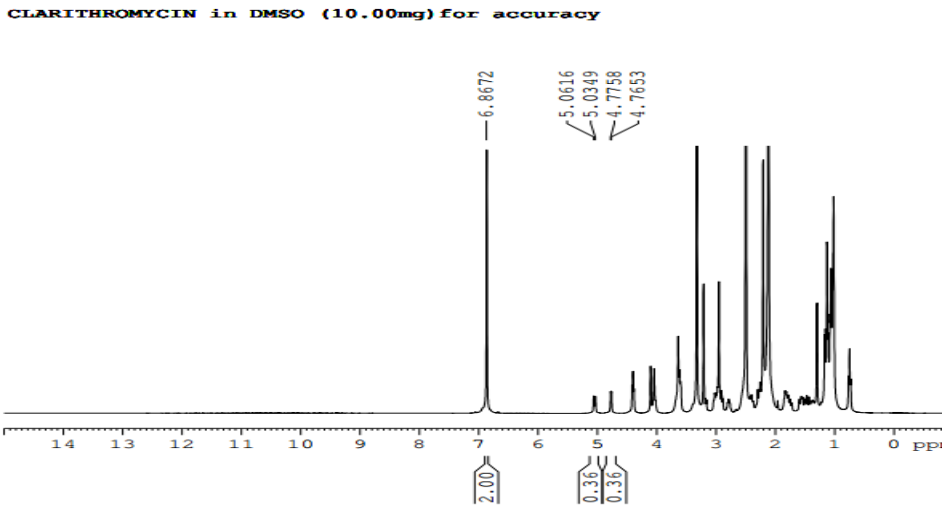


Fig. 5.8.4: Accuracy 10.00 mg Std + 0.6 ml stock.

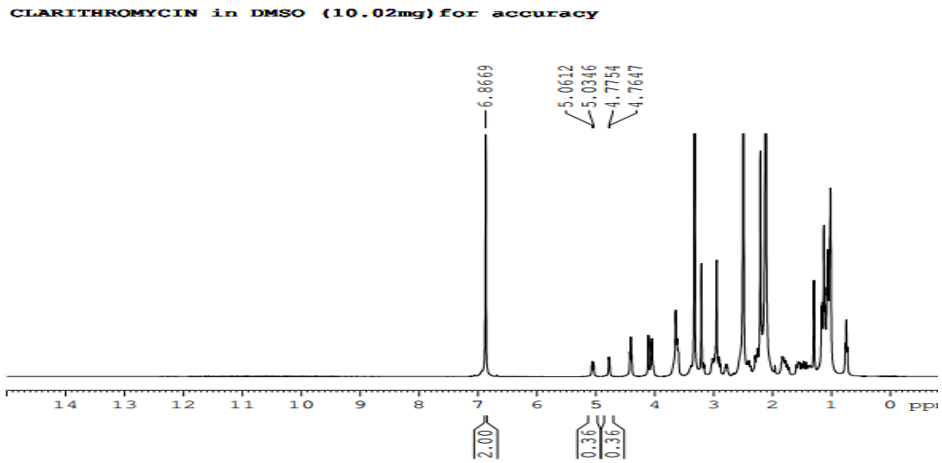


Fig. 5.8.5: Accuracy 10.02 mg Std + 0.6 ml stock.

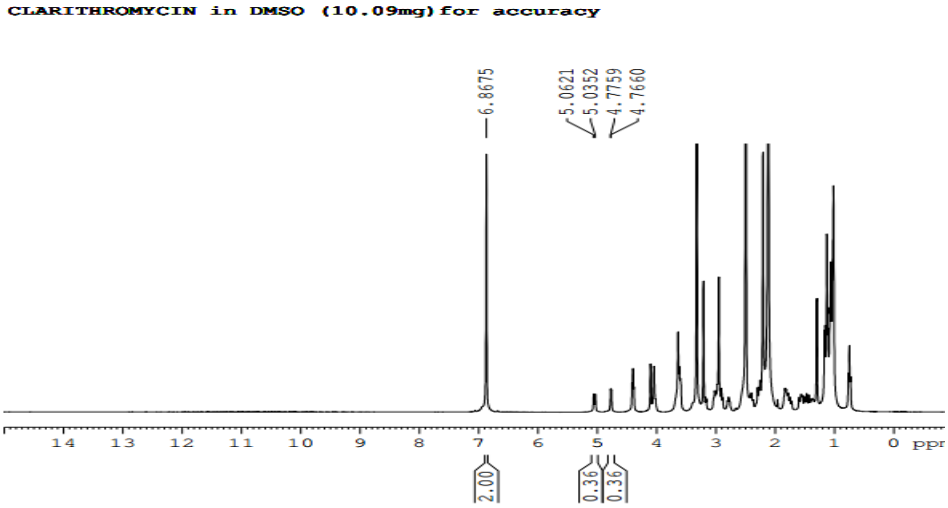


Fig. 5.8.6: Accuracy 10.09mg Std + 0.6 ml stock.

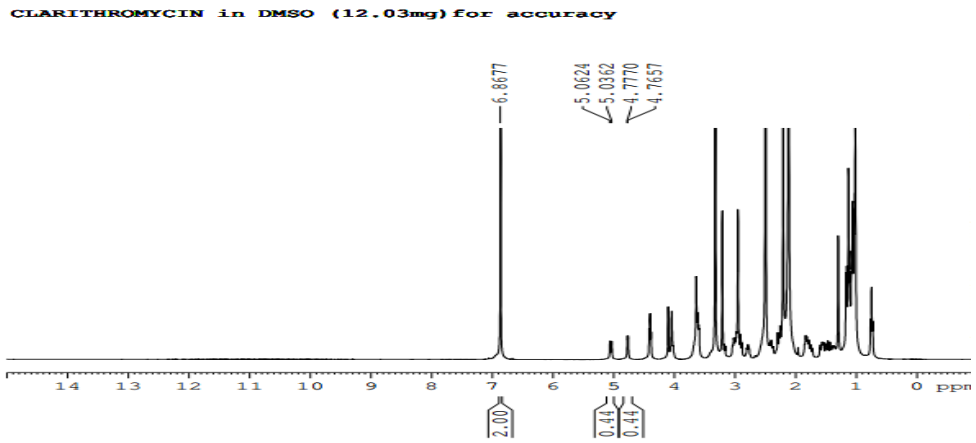


Fig. 5.8.7: Accuracy 12.03 mg Std + 0.6 ml stock.

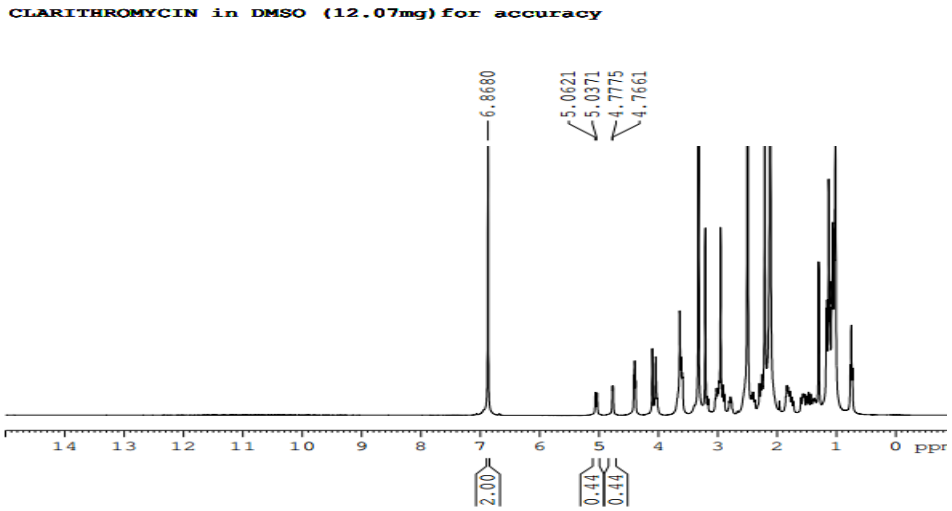


Fig. 5.8.8: Accuracy 12.07 mg Std + 0.6 ml stock.

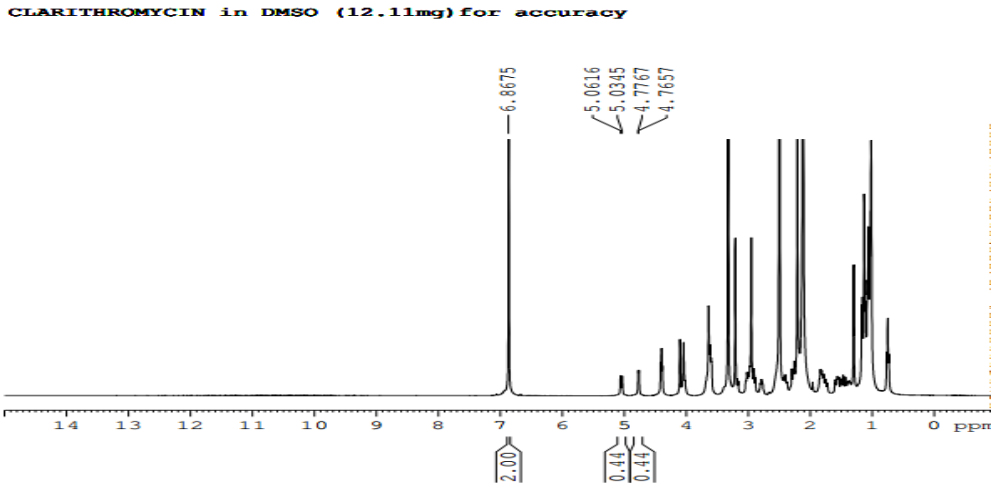


Fig. 5.8.9: Accuracy 12.11 mg Std + 0.6 ml stock.

5.2.6 Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in procedural parameters listed in the procedure documentation and provide an indication of its suitability during normal usage. The robustness of the method was evaluated by varying two parameters independently: (1) The number of scans (64 scans±16) and (2) The internal standard amount (20% variation) (10±2.0 mg). From the results obtained as per Table, running the experiment using a different number of scans such as 48 or 80 rather than 64 did not affect the measurement. A variation of 20% in internal standard amount also did not appreciably change the measured amount of drug.

Thereby, this method is quite robust in terms of the above-mentioned parameters.

Table 5.5.1: Tabular representation of the robustness parameter.

Parameter	Change	Found drug in mg	%Assay (As such)	%Difference
Number of scan	48	9.83	97.81	0.0
	64	9.83	97.81	NA
		9.83	97.81	0.0
		Mean	97.81	
		SD	0	
		%RSD	0	
Internal standard	5.05	8.19	102.8	0.45
	7.5	10.59	100.56	NA
	9.5	13.34	101.13	0.37
		Mean	101.50	
		SD	0.95	
		%RSD	0.93	

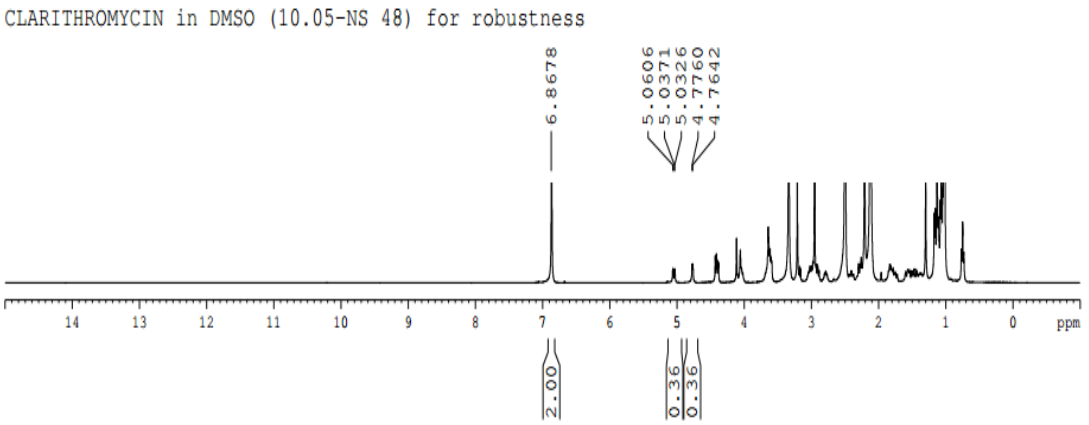


Fig. 5.9.1: Robustness 10.05 mg Std + 0.6 ml stock NS 48.

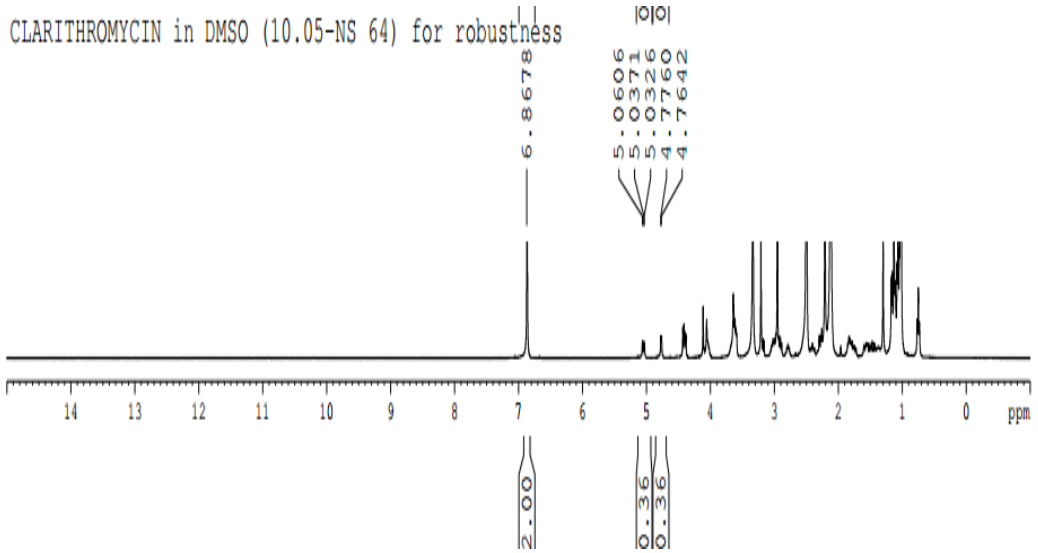


Fig. 5.9.2: Robustness 10.05 mg Std + 0.6 ml stock NS 64.

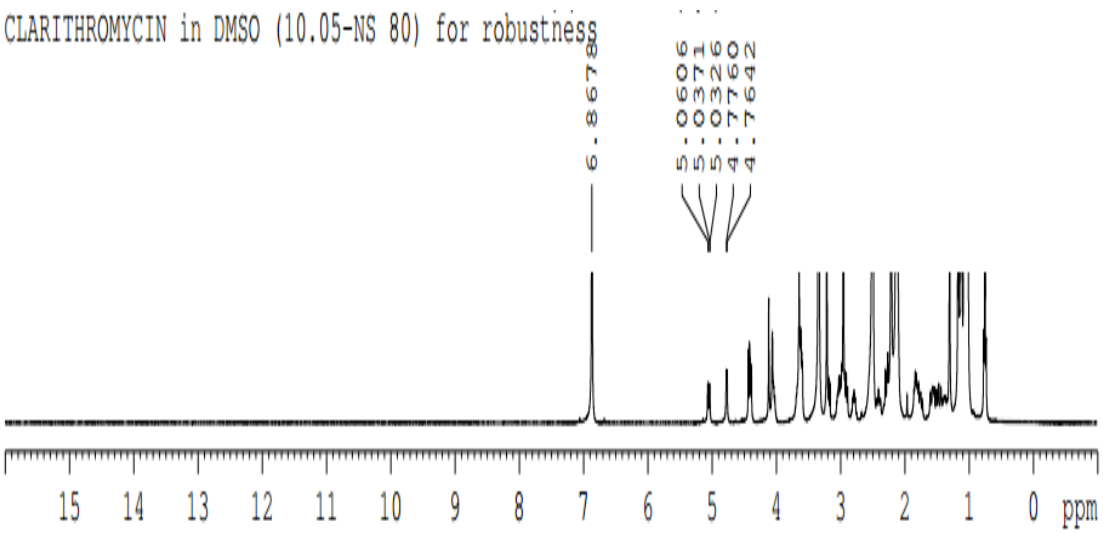


Fig. 5.9.3: Robustness 10.05 mg Std + 0.6 ml stock NS 80.

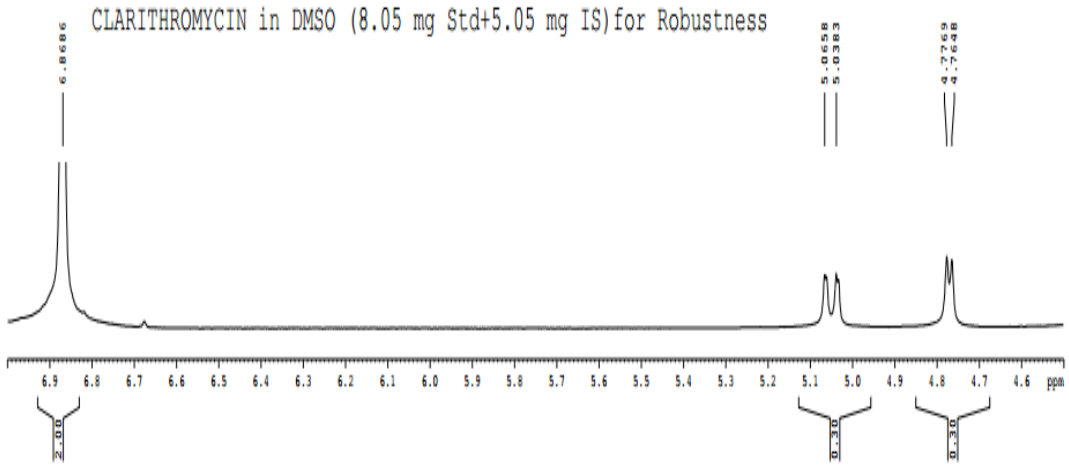


Fig. 5.9.4: Robustness 8.05 mg Std+5.05 mg IS in 0.6 ml DMSO.

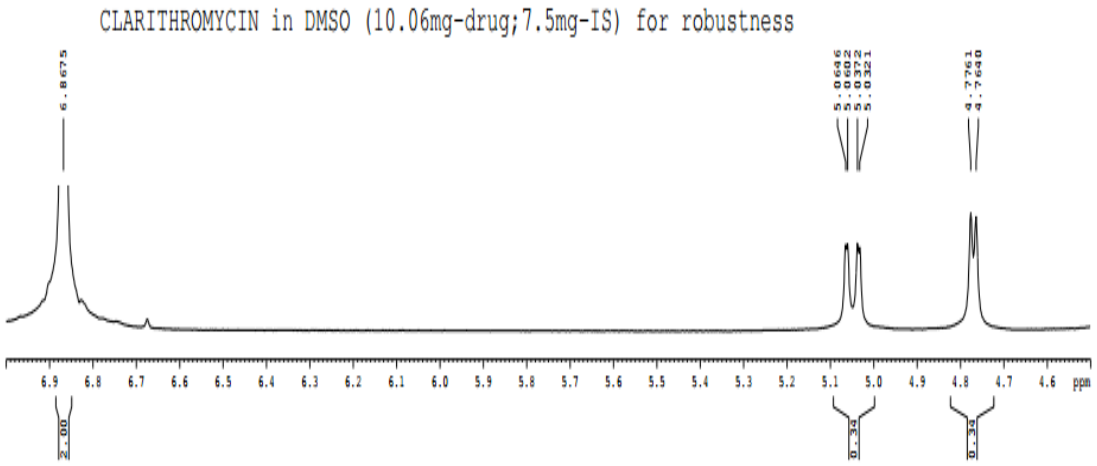


Fig. 5.9.5: Robustness 10.06 mg Std+7.5 mg IS in 0.6 ml DMSO.

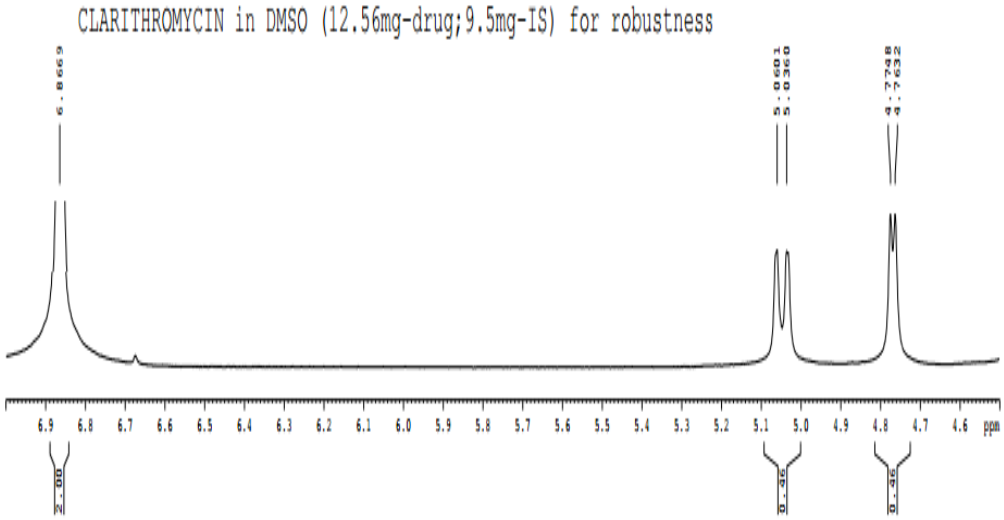


Fig. 5.9.6 Robustness 12.56 mg Std+9.5 mg IS in 0.6 ml.

5.4 Validation report summary of clarithromycin.

S. no.	Parameter	Experiment	Discussion	Acceptance criteria
1	Specificity	Interference to the IS and analyte proton	No interference at 6.87 ppm	No interference should be observed at IS peak and analyte proton.
2	Linearity	Coefficient of correlation (r)	0.999	≥ 0.99
3	Precision	Precision of six replicate samples prepared.	The relative standard deviation for six replicate preparations was found to be 0.45.	The relative standard deviation for six replicate preparations should be not more than 2.0%.
4	Reproducibility (Intermediate Precision)	Analyst to analyst	The %RSD for % assay obtained from 6 precision samples was	The %RSD for % assay obtained from 6 precision samples should be not

			found to be 0.82.	more than 2.0%.
5	Accuracy	Accuracy level 80%-120% and there triplicate	The %RSD for % assay obtained from 6 precision samples was found to be 0.74.	The %RSD for % assay obtained from 6 precision samples should be not more than 2.0%.
6	Robustness	1] Change in no. of scans(64±16)	The % RSD for % assay obtained from robustness study was found 0.00.	The % RSD for % assay obtained from robustness study should be not more than 2.0%.
		2] Variation in internal standard amount(20%)(10±2)	The % RSD for % assay obtained from precision and robustness study was found 0.93.	The % RSD for % assay obtained from robustness study should be not more than 2.0%.

CONCLUSION

The Assay method validation performed on drug substance Clarithromycin gives specific, precise, linear and accurate results for the method. The Assay method for Clarithromycin is also reproducible. Hence the assay method for Clarithromycin be used for routine analysis.

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