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ORIGINAL ARTICLE

RP-HPLC Method Development and Validation for Simultaneous Estimation of Cefuroxime and Linezolid in Bulk and Pharmaceutical Dosage Form

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ABSTRACT
RP-HPLC Method Development and Validation for Simultaneous Estimation of Cefuroxime and Linezolid in Bulk and Pharmaceutical Dosage form. A High performance liquidchromatograph WATERS, software: Empower 2, 2695 separation module, 996 PDA detector, using Phenomenex Luna C_{18} (4.6mm x 150mm, 5µm, Make: Waters) or equivalent column, with mobile phase composition of Acetonitrile: Phosphate Buffer (pH-6.8) [70:30 % (v/v)] was used. The flow rate of 1ml/min and effluent was detected at 230 nm. The retention time of Cefuroxime and Linezolid was found to be 2.813 min and 3.886 minutes respectively. Linearity was observed over concentration range of 25-125 µg ml-1 for Cefuroxime and 30-150 µg ml-1 for Linezolid respectively. The accuracy of the proposed method was determined by recovery studies and the Cefuroxime was found to be 99.8 % and Linezolid was found to be 99.4 % respectively. The proposed method isapplicable to stability studies and routine analysis of Cefuroxime and Linezolid in bulk and pharmaceutical formulations. The proposed method was validated for various ICH parameters like linearity, limit of detection, limits of quantification, accuracy, precision, range and specificity.
Key Words: *Cefuroxime, Linezolid, RP-HPLC, Robustness and ICH Guidelines.*

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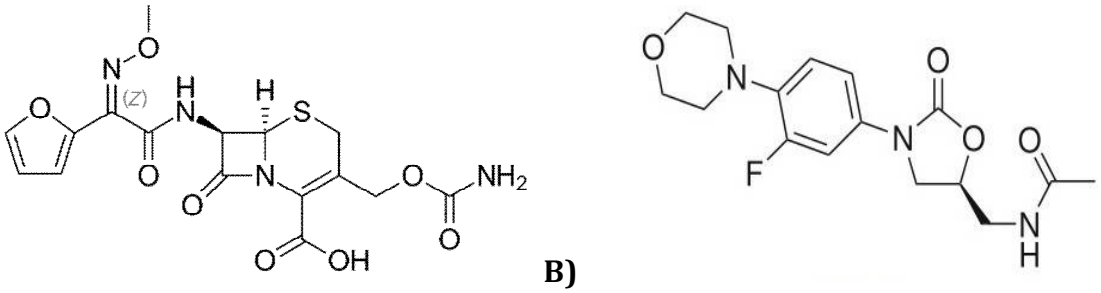
INTRODUCTION

Cefuroxime (CF) is a second-generation cephalosporin that contains the classic β-lactam ring structure. Cefuroxime is an ester prodrug of cefuroxime, which is rendered more lipophilic by esterification of carboxyl group of the molecule by the racemic 1- acetoxyethyl bromide, thus enhancing absorption. The absorbed ester is hydrolysed in the intestinal mucosa and in portal circulation. Products of hydrolysis are active cefuroxime, acetaldehyde and acetic acid. Cefuroxime is chemically (1RS)-1-[(acetyl) oxy] ethyl-(6R, 7R)-3-(carbamoyloxy) ethyl]-7-[(Z-2-furan- 2yl)-2- (methoxyimino) acetyl) amino]-8-oxo-5-thia-1-azabicyclo- (4.2.0)-oct-2-ene-2-carboxylate. It is used as an antibiotic for the treatment of many type of bacterial infections such as bronchitis, sinusitis, tonsillitis, ear infections, skin-infections, urinary tract infections[1-3].

Linezolid (LNZ) is chemically (*S*)-*N*-({3-[3-fluoro-4-(morpholin-4-yl) phenyl] - 2-oxo-1,3-oxazolidin-5-yl}methyl)acetamide. It is member of oxazolidinone class. It is used for the treatment of serious infection caused by Gram positive bacteria that resistance to other antibiotics. The main uses are infections of the skin and pneumonia although it may be use for a variety of other infections. Oxazolidinone bind to the 50S subunit of the prokaryotic ribosome, preventing it from complexing with the 30S subunit, mRNA, initiation factors and formylmethionyl- tRNA. The net result is to block assembly of a functional initiation complex for protein synthesis, thereby preventing translation of the mRNA [4-6].

Literature review reveals that numbers of individual analytical methods available for estimation of Cefuroxime and Linezolid in their individual dosage forms. But HPLC method has been not reported for simultaneous estimation of Cefuroxime and Linezolid in solid dosage forms. So it is develop RP-HPLC method for simultaneous estimation of Cefuroxime and Linezolid in Pharmaceutical Dosage Form and validated as per ICH guideline.



A) **B)**

Fig. 1: Structure of A) Cefuroxime and B) Linezolid.

MATERIAL AND METHODS

Instruments

Table 1 represents the list of instruments used in the present research.

Table 1: Instruments used

SL.No	Instrument	Model
1	HPLC	WATERS, software: Empower 2, 2695 separation module. 996 PDA detector.
2	UV/VIS spectrophotometer	Labindia UV
3	pH meter	Lab India
4	Weighing machine	Sartorius
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Enertech

Chemicals

Table 2 represents the list of chemicals used in the present research.

Table 2: chemicals used

S.No	Chemical	Brand names
1	Cefuroxime	Hetero labs
2	Linezolid	Hetero labs
4	Water and Methanol for HPLC	LICHROSOLV (MERCK)
5	Acetonitrile for HPLC	Merck
6.	Anhydrous di hydrogen phosphate	Finar chemicals
7	Citric acid	Finar chemicals

HPLC method development

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Orthophosphoric acid and Phosphoric acid (pH 3): Acetonitrile and Methanol: ACN and Methanol: Ammonium acetate Buffer with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Water in proportion 70:30 v/v respectively [7-9].

Optimization of Column

The method was performed with various columns like C18 column, X- bridge column, Xterra and C8 column. Hypersil C18 (4.6 x 150mm, 5µm, Make: Waters) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Optimized chromatographic conditions

Instrument used :Waters HPLC with auto sampler and PDA detector 996 model.
Temperature : Ambient
Column : Hypersil C18 (4.6 x 150mm, 5µm, Make:Waters)
Mobile phase : Acetonitrile: Water: Methanol (60:20:20v/v)
Flow rate : 1 ml per min
Wavelength : 230 nm
Injection volume : 10 µl

Run time : 7 min.
Optimized chromatogram, blank, System suitability parameters are shown in the figure and the results are shown in Table.

Preparation of mobile phase

Accurately measured 700 ml (70%) of Acetonitrile and 300 ml of Water (30%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Validation

Method Precision

The standard and sample solutions of 75µg/ml of Cefuroxime, 90µg/ml of Linezolid were injected for five times and the peak areas were recorded. The mean and percentage relative standard deviation were calculated from the peak areas [7-11].

Intermediate Precision/Ruggedness

The standard and sample solutions of containing concentrations were 75µg/ml of Cefuroxime and 90µg/ml of Linezolid. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits [12-14].

Accuracy

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the Amount found and Amount added for Cefuroxime, Linezolid and calculate the individual recovery and mean recovery values. These solutions were filtered through 0.45µ membrane and then each concentration; three replicate injections were made under the optimized conditions. Recorded the chromatograms and measured the peak responses.

Linearity

Preparation of stock solution

Accurately weigh 10 combination tablets crush in mortar and pestle and transfer equivalent to 10 mg of Cefuroxime, Linezolid (marketed formulation-dose of Cefuroxime is 500mg, Dose of Linezolid is 600mg in combination tablet) sample into a 100 mL clean dry volumetric flask add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent[14-16]. (Stock solution 100 ppm)

Preparation of Level – I (25ppm of Cefuroxime & 30ppm of Linezolid)

0.3ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (50ppm of Cefuroxime & 60ppm of Linezolid)

0.6ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (75ppm of Cefuroxime & 90ppm of Linezolid)

0.9ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (100ppm of Cefuroxime & 120ppm of Linezolid)

1.2ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (125ppm of Cefuroxime & 150ppm of Linezolid)

1.5ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

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.

Robustness

Effect of Variation of flow

The sample was analyzed at 0.8 ml/min and 1.0 ml/min instead of 0.9 ml/min, remaining conditions are same. 10µl of the above sample was injected twice and chromatograms were recorded

Effect of Variation of mobile phase organic composition

The sample was analyzed by variation of mobile phase i.e. Acetonitrile: Water was taken in the ratio and 60:40, 80:20 instead 70:30, remaining conditions are same. 10µl of the above sample was injected twice and chromatograms were recorded.

RESULT AND DISCUSSION

Optimized trial conditions

Mobile phase : Acetonitrile: Water:Methanol (60:20:20v/v)
Column : Hypersil C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 230 nm

Column temp : Ambient
Sample Temp : Ambient
Injection Volume : 10 µl
Run time : 7 minutes

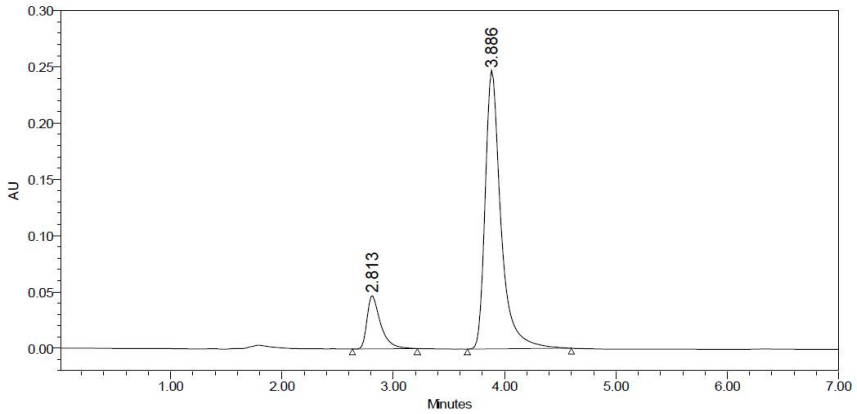


Fig. 2: Chromatogram for optimized trail.

System suitability

Table 3: Results of system suitability parameters for Cefuroxime and Linezolid

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Cefuroxime	2.816	572745	66043		1.5	2642
2	Linezolid	3.893	3423737	340922	4.5	1.5	4153

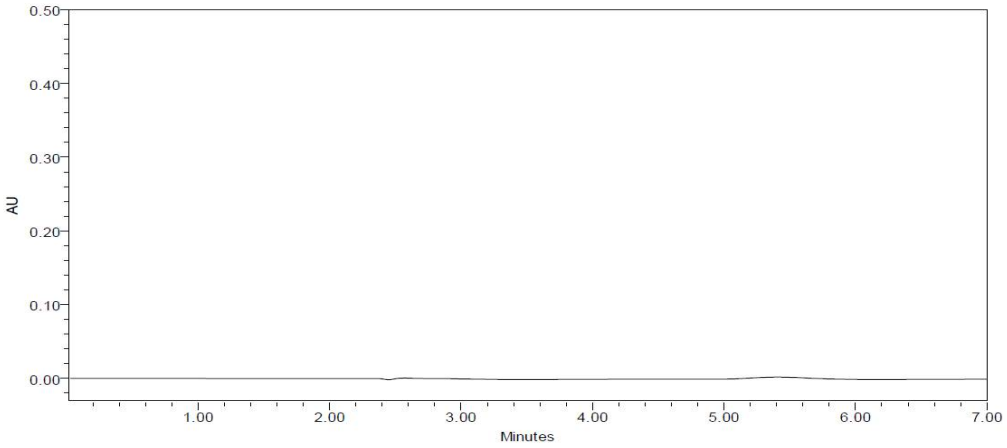


Fig. 3: Chromatogram showing blank (mobile phase preparation)

Assay

The retention time of Cefuroxime and Linezolid was found to be 2.860mins and 3.886mins respectively. The % purity of Cefuroxime and Linezolid in pharmaceutical dosage form was found to be 99.6% and 99.8% respectively.

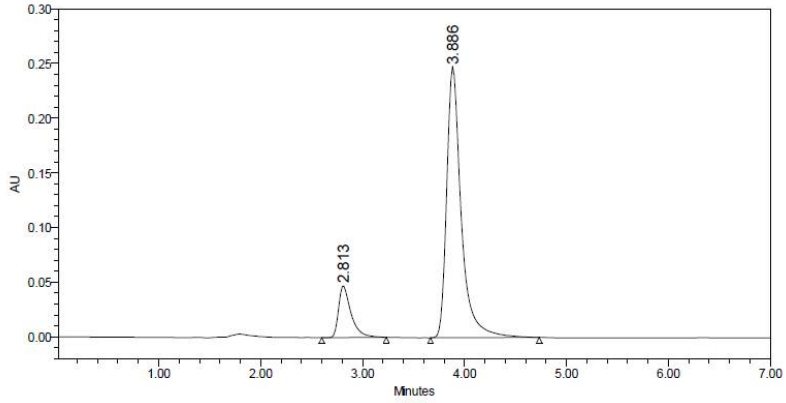


Fig. 4: Chromatogram showing assay of standard injection

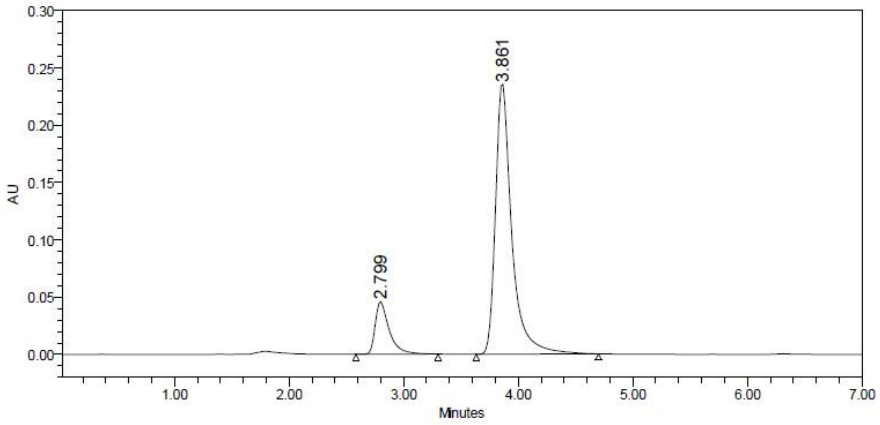


Fig. 5: Chromatogram showing assay of sample injection

Table 4: Showing assay results

S.No	Name of compound	Label claim	Amount taken (from combination tablet)	%purity
1	Cefuroxime	500mg	499.8	99.6 %
2	Linezolid	600mg	599.9	99.8%

Validation

Precision

Precision of the method was carried out for both sample and standard solutions as described under experimental work. The corresponding chromatograms and results are shown below [13].

Table 5: Results of method precession for Cefuroxime and Linezolid.

Cefuroxime						
Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Cefuroxime	2.808	368013	46097	4536	1.6
2	Cefuroxime	2.808	372552	46244	4236	1.6
3	Cefuroxime	2.808	367873	46092	4565	1.6
4	Cefuroxime	2.808	375555	46312	4682	1.6
5	Cefuroxime	2.808	374843	46275	4521	1.6
6	Cefuroxime	2.808	368013	46097	4561	1.6
Mean			371767			
Std. Dev			3663.5			
% RSD			0.9			
Linezolid						
1	Linezolid	3.880	2321302	241739	4641.3	1.5
2	Linezolid	3.880	2308016	241530	4632.2	1.5
3	Linezolid	3.880	2326058	241796	4621.6	1.5
4	Linezolid	3.880	2334897	241910	4695.3	1.5
5	Linezolid	3.880	2326143	241799	4691.7	1.5
6	Linezolid	3.880	2324512	241639	4685.1	1.5
Mean			2323283			
Std. Dev			9845.8			
% RSD			0.42			

Table 6: Results of Intermediate precision for Cefuroxime and Linezolid

	Cefuroxime			Linezolid		
Sno	Rt	Area	USP Tailing	Rt	Area	USP Tailing
1	2.808	377409	1.6	3.882	2268108	1.5
2	2.808	371977	1.6	3.882	2275775	1.5
3	2.808	376191	1.6	3.882	2254168	1.5
4	2.808	372169	1.6	3.882	2285916	1.5
5	2.808	378930	1.6	3.882	2296220	1.5
6	2.808	378624	1.6	3.882	22984261	1.5
Mean		375335			2276037	
Std. Dev		3132.9			16171.8	
% RSD		0.83			0.71	

Accuracy

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated [15].

Table 7: Accuracy data for Cefuroxime and Linezolid

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
Cefuroxime					
50%	569325	5	4.9	98%	99.8%
100%	753538	10	10.1	101%	
150%	955999	15	15.1	100.6%	
Linezolid					
50%	3441832	5	4.9	98%	99.4%
100%	4517559	10	10.1	101%	
150%	5738638	15	14.9	99.3%	

Linearity:

The linearity range was found to lie from 25µg/ml to 125µg/ml of Cefuroxime, 30µg/ml to 150µg/ml of Linezolid and chromatograms are shown below [7-9].

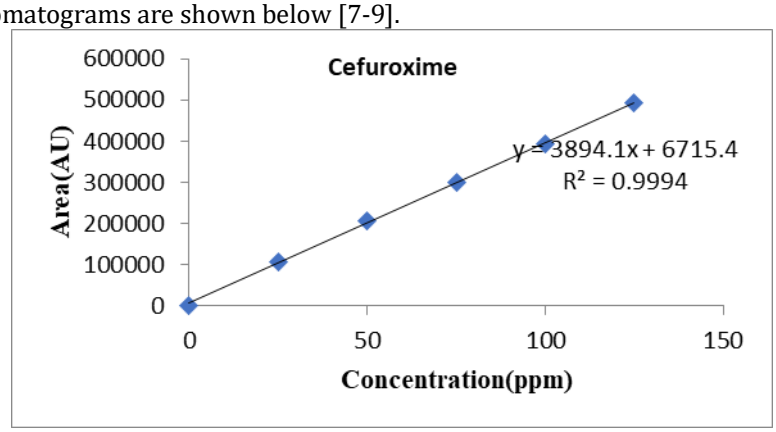


Fig. 6: Calibration graph for Cefuroxime

Table 8: Linearity Results for Cefuroxime and Linezolid

S.No	Cefuroxime			Linezolid		
	Linearity Level	Concentration (ppm)	Area	Linearity Level	Concentration (ppm)	Area
1	I	25	108407	I	30	606125
2	II	50	206978	II	60	1208367
3	III	75	299892	III	90	1804843
4	IV	100	393459	IV	120	2371642
5	V	125	491862	V	150	2885708
Correlation Coefficient			0.999			0.999

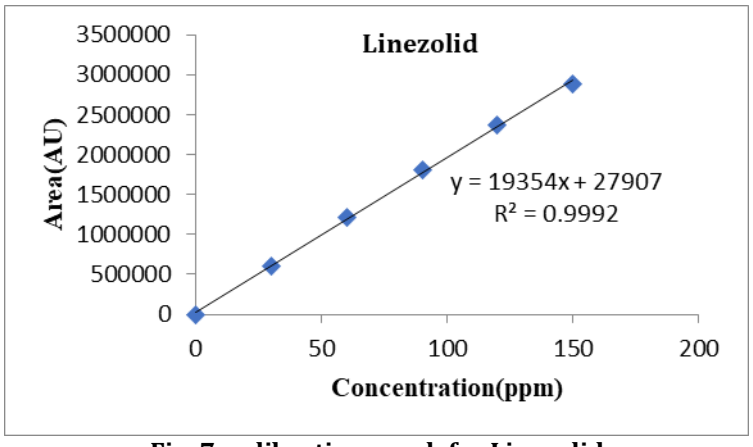


Fig. 7: calibration graph for Linezolid

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. It was determined from the formula:

$$\mathbf{LOD = 3.3 \times \sigma / s}$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Cefuroxime and Linezolid LOD values were found to be 1.6µg/ml and 5.6µg/ml, respectively.

Quantitation limit

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined. It was determined from the formula:

$$\mathbf{LOQ = 10 \times \sigma / S}$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Cefuroxime and Linezolid LOQ values were found to be 4.9µg/ml and 17.0µg/ml, respectively.

Robustness

The standard and samples of Cefuroxime and Linezolid were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table 9: System suitability results (at variable flow) for Cefuroxime and Linezolid.

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
Cefuroxime	0.9	2741.14	1.71
	1.0	2423.3	1.6
	1.1	2543.21	1.58
Linezolid	0.9	4162.06	1.57
	1.0	4641.3	1.5
	1.1	3921.45	1.49

Table 10: System suitability results (change in mobile phase) for Cefuroxime and Linezolid.

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
Cefuroxime	10% less	2980.49	1.60
	*Actual	2423.3	1.6
	10% more	2423.52	1.64
Linezolid	10% less	9407	1.01
	*Actual	4641.3	1.5
	10% more	4457.17	1.44

CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tools of the analysis. The estimation of Cefuroxime and Linezolid was done by RP-HPLC. The mobile phase was optimized with consists of Acetonitrile: Water. Acetonitrile mixed in the ratio of 70:30 % v/ v. A Hypersil C_{18} column (4.6 x 150mm, 5µm, Make: Waters) or equivalent chemically bonded to porous silica particles was used as stationary phase. The solutions were chromatographed at a constant flow rate of 1ml/min. the linearity range of Cefuroxime and Linezolid were found to be from 25-125ppm, 30-150ppm respectively. Linear regression coefficient was not more than 0.999, 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery of Cefuroxime is 99.6 and Linezolid is 99.8%. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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