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Original Article

STABILITY INDICATING SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF NICARDIPINE IN THE PRESENCE OF ITS ALKALINE INDUCED DEGRADATION PRODUCTS

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ABSTRACT

Objective: Derivative, ratio spectra derivative and ratio difference spectrophotometric methods were developed and validated for simultaneous determination of Nicardipine (NIC) in the presence of its alkaline induced degradation products.

Methods: In these methods the overlapped spectra of NIC and its alkaline induced degradation products were well resolved by measuring the amplitudes of first derivative (D^1) spectra and the second derivative (D^2) at 382.3 and 239 nm, respectively. NIC was determined by ratio spectra derivative by measuring the amplitude at 244 nm. The ratio difference spectrophotometric method was developed in which the difference between amplitudes at 237.5 nm and 260 nm of the ratio spectra were recorded. The linearity range for the applied methods was 2-18 µg/ml.

Results: All the developed methods were validated according to ICH Guidelines, NIC was determined with acceptable accuracy and precision.

Conclusion: These methods were suitable as stability indicating methods for the determination of NIC in the presence of its alkaline induced degradation products either in bulk powder or in a pharmaceutical formulation. Statistical analysis of the results with those obtained by applying a reported method has been carried out revealing high accuracy and good precision.

Keywords: Nicardipine, Spectrophotometry, Pharmaceutical preparations, Stability indicating, derivative, ratio derivative and ratio difference

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INTRODUCTION

Nicardipine hydrochloride (NIC) was designated as 2-[Benzyl (methyl) amino] ethyl methyl 1, 4-dihydro-2, 6-dimethyl-4-(3-nitrophenyl) pyridine-3, 5-dicarboxylate hydrochloride [1]. It is a calcium channel antagonist of dihydropyridine class. It is currently used for the management of angina pectoris and also used in the treatment of hypertension [2].

Few methods have been reported for determination of NIC: these methods included spectrophotometry [3-6], voltammetry [7], high-performance liquid chromatography [8-10], liquid chromatography–mass spectrometry [11-13], and capillary electrophoresis [14]. A study on forced degradation and degradation kinetics was also reported [15, 16]. Most of the previously reported work was focused on the determination of NIC in biological fluids.

Thus, our aim was to develop and validate simple spectrophotometric stability indicating methods for the determination of NIC in the presence of its alkaline induced degradation products. These methods include first derivative, second derivative, derivative ratio and ratio difference.

Experimental

Instruments

Spectrophotometer, UV–VIS Spectrophotometer (1650 Shimadzu, kyoto, Japan) with UV-PC personal spectroscopy software version 3.7 (Shimadzu) with quartz cells of 1 cm path length. The spectral bandwidth was 2 nm and the wavelength scanning speed was 2800 nm/min. IR Spectrometer, Shimadzu 435 (Kyoto, Japan), sampling was prepared in disc form with potassium bromide. Mass spectrophotometer, MS-QB 1000 EX, Finnigan Nat (USA).

Chemicals and reagents

Pure NIC, batch no. C 100921585, its purity was found to be 100.23% according to the reported method [6] was purchased from (SIGMA Aldrich, USA). Loxen® tablets, batch no. T0109, were manufactured by Novartis pharm S. A. S. France and purchased from international market (France). Methanol used was of HPLC grade (SIGMA Aldrich, USA). Other chemicals were of analytical grade. Chloroform and hydrochloric acid (Al-Gomhoria, Egypt), Sodium hydroxide (Adwic, Egypt)

Solutions

Standard solution

A standard stock solution of NIC (0.1 mg/ml) was prepared by dissolving accurately weighed 10 mg of NIC in 100-ml volumetric flask using methanol as solvent.

Preparation and identification of degradation products

Preparation of alkaline induced degradation products was performed by transferring accurately weighed 50 mg of pure NIC in a standard 250-ml conical flask, these dissolving in 50-ml methanol, 50-ml of 0.5N NaOH were added, and the solution was left for 7 h in an oven at 100°C. The solution was allowed to cool at room temperature then neutralized by 0.5N hydrochloric acid, evaporated under vacuum nearly to dryness, transferred, dissolved and completed to volume with methanol in 50-ml volumetric flask. For the purpose of identification, the solution was spotted on to preparative TLC-plates, developed using chloroform: methanol (9:1, v/v). Two bands were separated, scratched, dissolved in methanol, filtered, evaporated to dryness and the dried powder of each of the two degradation products were subjected to MS and IR analysis.

Spectrophotometric methods

Spectral characteristics

The absorption spectra of standard solution NIC and its degradation products were recorded over the range 200-400 nm using methanol as blank.

Construction of calibration curves

Aliquots of NIC standard solution were accurately transferred into a set of 10-ml volumetric flask and the volume was completed with

methanol to reach a final concentration range of (2.00-18.00 µg/ml). The absorption spectra of the resulting solution were measured in the range of 200-400 nm using methanol as blank.

First derivative (D^1) method

D^1

Second derivative (D^2) method

D^2curves of the stored spectra were calculated using (Δλ=4.00, scale=100), the peaks amplitude at 239 nm were plotted versus the corresponding concentrations, and the regression equation was computed.

Derivative ratio method (DD^1)

The stored spectra were divided by the absorption spectrum of 4.00 µg/ml of NIC degradation products; the obtained ratio spectra were subjected to D^1 calculation using (Δλ=4.00, scale=10). The peak amplitudes at 244 nm were plotted versus the corresponding concentration, and a regression equation was computed.

Ratio difference method (RD)

The stored spectra were divided by the absorption spectrum of 4.00 µg/ml of NIC degradation products. The differences between the amplitude at 237.4 nm and 260 nm of the obtained ratio spectra were calculated, plotted versus the corresponding concentration of NIC, and the regression equation was computed.

Assay of laboratory prepared mixtures

Different mixtures of NIC and NIC degradation products were prepared by accurately transferring aliquots from their solutions to prepare mixtures containing from 10%-80% of NIC degradation products. Then the procedure under each method was followed.

Assay of pharmaceutical formulations

10 tablets of Loxen® tablets were weighed and finely powdered. Accurately weighed the amount of the powder, equivalent to 0.01g of NIC, was transferred into 100-ml volumetric flask , mechanically shaken for 20 min, filtered, and the volume was completed to obtain 0.1 mg/ml solution. The absorption spectra of the resulting solution were measured in the range of 200-400 nm and the procedure of each method was followed. The concentration of NIC was calculated by each of the proposed methods from its corresponding regression equation.

RESULTS AND DISCUSSION

The International Conference on Harmonization (ICH) guideline entitled "stability testing of new drug substances and products" required the stress testing to be carried to elucidate the inherent stability characteristics of the active substance. An ideal stability indicating method is the one that quantifies the standard drug alone and also resolved its degradation products [17].

The two ester groups attached to dihydropyridine ring in NIC have hydrolysed in alkaline medium resulted in the formation of two degradation products and methanol. The alkaline hydrolysis was achieved by heating NIC with 0.5 N NaOH at 60 °C for one hour [16].

Many trials were achieved for choice the condition of degradation of NIC, but the best results for complete degradation were achieved by heating NIC with 1 N NaOH at 100 °C for 7 h.

The focus of the present work was to develop a simple, accurate, specific and reproducible stability indicating spectrophotometric methods for the determination of NIC in its pure form and in pharmaceutical formulations in the presence of its alkaline induced degradation products.

Complete hydrolysis was achieved upon using 0.5 N NaOH for 7 h at 100 °C (fig. 1). The drug undergoes complete degradation into two degradation products and methanol.

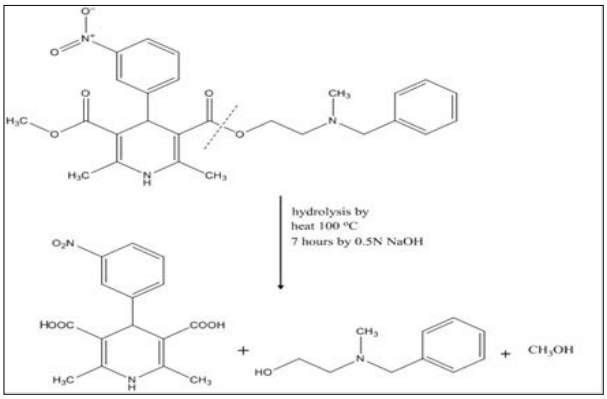


Fig. 1: Proposed scheme for preparing the degradation products of NIC

Separation of degradation products was achieved by application to preparative TLC plates using chloroform: methanol (9:1, v/v) as a developing solvent.

The separated degradation products were studied using MS and IR analyzes.

MS–analysis reveals parent peaks at 166.71 and 226.43 m/z, corresponding to the molecular weights of the two degradation products (fig. 2). By the comparison of the these IR-spectra of NIC with that the degradation products, the characteristic band at 1735-1750 corresponding to the C=O of the ester groups shown in (fig. 3), has been disappeared in the IR-spectra of degradation products (fig. 4 A, B) with the appearance of two new peaks at 3400, 1700 corresponding to hydroxyl and carboxylic groups, respectively.

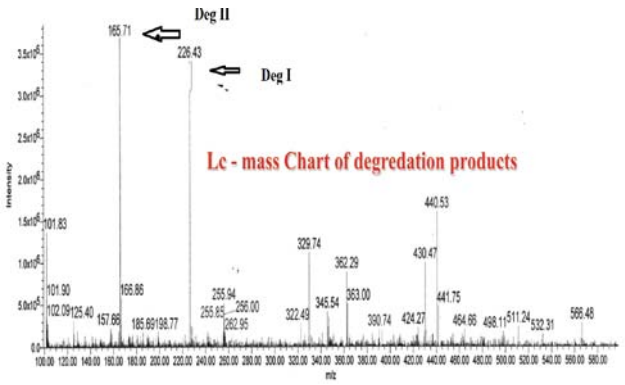


Fig. 2: Mass chart of the degradation products of NIC

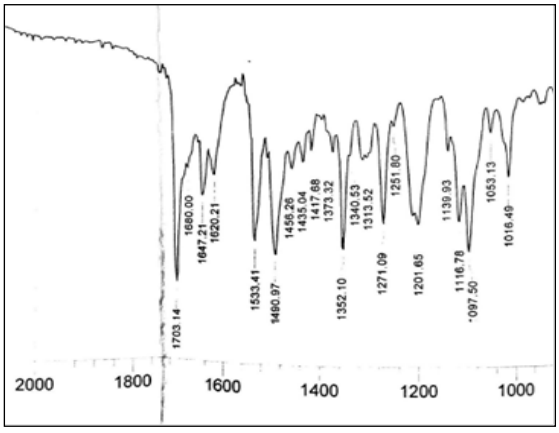
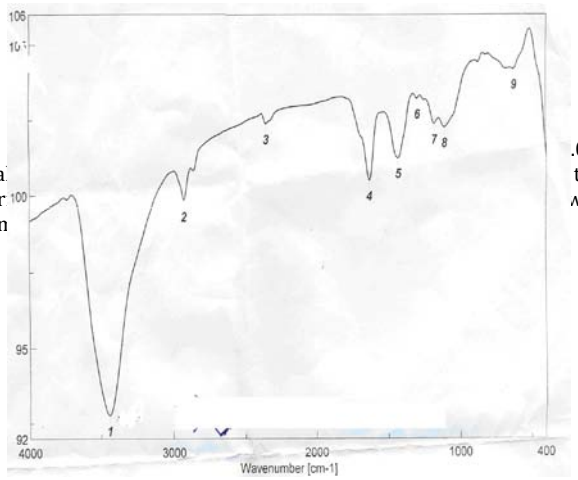
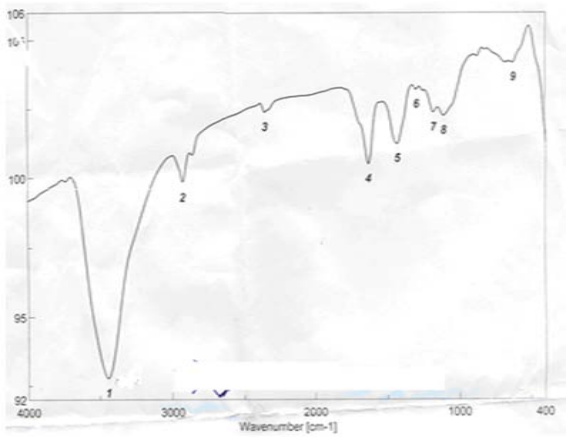


Fig. 3; IR chart of NIC



(A)



(B)

Fig. 4(A, B): IR chart of degradation products

NIC and its degradation products spectra (fig. 5), showed spectral overlapped with the degradation products at wavelength 350 nm and 250 nm.

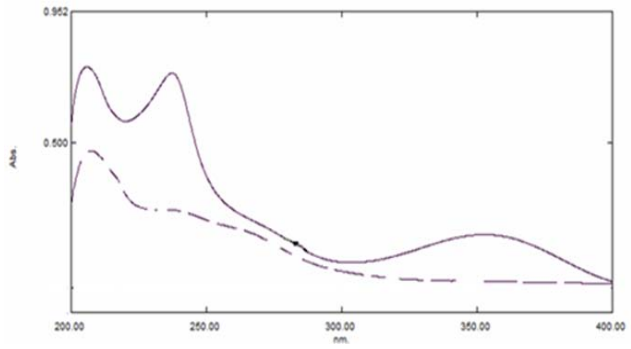


Fig. 5: Zero order absorption spectra of (10.00 µg/ml) of NIC (—) and (10.00 µg/ml) of the alkaline degradation products (----) using methanol as blank

First derivative method

In order to optimize D^1method, different smoothing and scaling factors were tested, where a smoothing factor Δλ= 4.00 and a scaling factor = 100 showed a suitable signal to noise ratio and the spectra showed good resolution. D^1 method was based on measuring the peak amplitude of NIC at 382 nm (fig. 6), (corresponding to zero-crossing of the degradation products). This method gave satisfactory results for NIC in the presence of up to 30% of its degradation products.

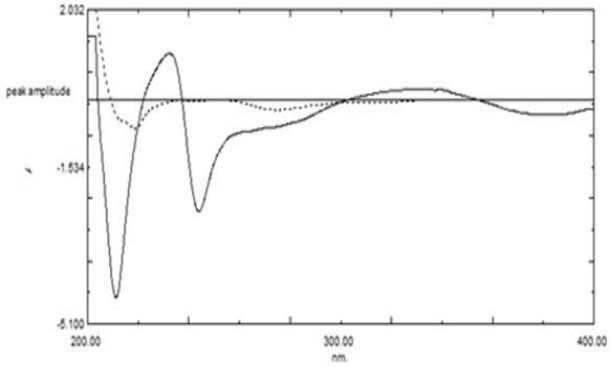


Fig. 6: First derivative spectra of 10.00 µg/ml of each of NIC (-) and degradation products (----) using methanol as blank

Second derivative method (D^2)

In order to optimize D^2 method, different smoothing and scaling factors were tested, where a smoothing factor Δλ= 4.00 and a scaling factor = 100 showed a suitable signal to noise ratio and the spectra showed good resolution. The peak amplitudes of D^2 spectrum of NIC at 239 nm (fig. 7), (corresponding to zero-crossing of the degradation products) was measured. This method could determine NIC in the presence of up to 80% of its degradation products.

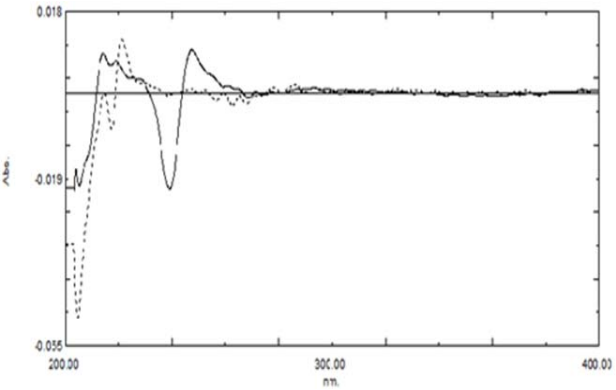


Fig. 7: Second derivative spectra of 10µg/ml of each of NIC (-) and the degradation products (----) in methanol

First derivative of ratio spectra (DD^1) method

In order to improve the selectivity of the analysis of NIC in the presence of alkaline induced degradation products, DD^1 spectrophotometric method was developed. The main advantage of the method is that the whole spectrum of the interfering substance was canceled. Accordingly, the choice of the wavelength for calibration was not critical as in derivative methods. In order to optimize DD^1 method, several divisors concentrations (2.00-16.00 µg/ml) were tried, the best result was obtained upon using 4.00 µg/ml of the degradation products. Different smoothing and scaling factors were tested, where a smoothing factor Δλ = 4.00 and a scaling factor = 10 were suitable to enlarge the signal of NIC to facilitate its measurement and to diminish the error (fig. 8,9). DD^1 values showed good linearity and reproducibility at 244 nm.

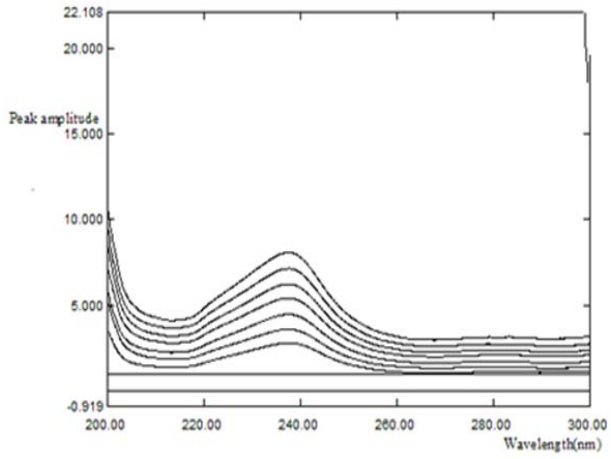


Fig. 8: Ratio spectra of NIC 6-18µg/ml (—) using 4 µg/ml of degradation products as a divisor

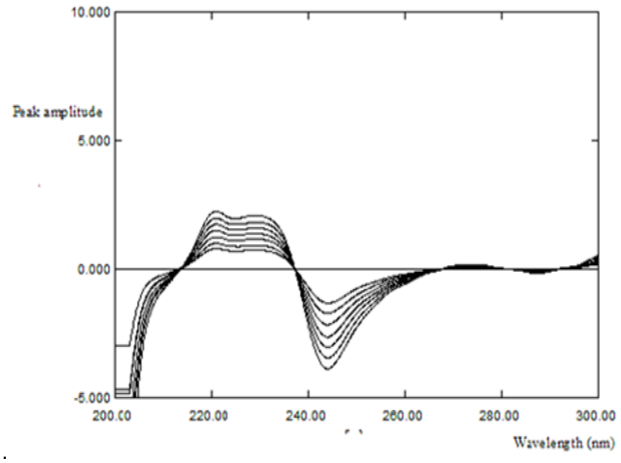


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Fig. 9: First derivative of ratio spectra of NIC (2.00–18.00 µg/ml) using 4 µg/ml of Degradation alkaline products as a divisor

Ratio difference method (RD)

The amplitude difference between two points on the ratio spectra of a mixture is directly proportional to the concentration of the component of interest; independence of the interfering component was the basic principle of the difference ratio method [18].

The spectra of NIC were scanned. The laboratory prepared mixtures were measured and divided by the absorption spectra of 4.00µg/ml alkaline induced degradation products, where they obtained ratio spectra were recorded. The calibration curve for NIC was constructed by plotting the difference between the amplitudes of obtained ratio spectra at 237.4 nm and 260 nm versus the corresponding concentrations of NIC.

Method validation

Method validation was performed according to ICH guidelines for the proposed methods as follows:

Range and linearity

The linearity of the method was evaluated by processing 6 points calibration curve on 3 different days. A linear relationship was obtained in the range of 2.00-18.00 µg/ml. A linear least-square regression analysis was conducted to determine slope, intercept, and coefficient of determination to demonstrate linearity of the method. The linear regression analysis data are summarized in table 1.

Limits of detection and quantitation

The limit of detection (LOD) and limit of quantification (LOQ) was calculated as the ratio of 3.3 and 10 standard deviations of regression residuals, respectively, and the slope.

Accuracy

The procedure under the study of linearity was repeated three times for determination of six different concentrations of pure NIC. The accuracy expressed as percentage recoveries were shown in table 1.

Precision

The precision was determined by analysis of 3 different concentrations of pure NIC (6.00, 8.00, 10.00 µg/ml) within the linearity range for NIC. Both intraday precision and inter-day precision were determined. The results are presented as RSD% in table 1.

The proposed methods successfully used for the determination of NIC in laboratory prepared mixtures containing different ratios of the drug and its degradation products. The mean recovery percentages and standard deviations were illustrated in table 2.

The suggested methods were successfully applied for the analysis of NIC in a pharmaceutical formulation. The accuracy of the method was further assessed by applying the standard addition technique; good results were obtained and shown in table 3.

The results obtained by applying the proposed methods were compared to those obtained by apply the reported HPLC method [6], no significant difference was found with respect to both accuracy and precision as shown in table 4.

Table 1: Assay parameters and validation sheet for determination of NIC by the proposed method

Parameters	D^1 method*	D^2 method*	DD^1 method*	RD *
Range (µg/ml)	2.00-18.00	2.00-18.00	2.00-18.00	2.00-18.00
Slope	0.02	0.04	0. 21	0.02
Intercept	0.03	0.03	0.02	0.02
Correlation coefficient	0.99	0.99	0.99	0.99
Accuracy mean±SD	100.16±1.24	100.04±1.42	98.98±1.42	99.80±1.77
RSD%[a]*	1.28	1.47	0.99	1.04
RSD% [b]*	0. 84	0.80	1.13	0.98
LOD (µg/ml)	0.21	0.24	0.27	0.14
LOQ (µg/ml)	0.65	0.74	0.84	0.43

RSD %[a]* Interday and RSD% [b]*Intraday of samples of concentrations (6, 8, 10 µg/. ml) for D^1, D^2, DD^1, and RDSM method, *D^1: First derivative method, *D^2: Second derivative method, *DD^1: Derivative ratio method, *RD: Ratio difference spectrophotometric method, The sample sizes are three separate determinations.

Table 2: Determination of NIC in laboratory prepared mixtures by the proposed spectrophotometric methods

Mixture No.	% of degradation products	D^1 method	D^2 method	DD^1 method	RD
1	10	101.20	99.02	100.86	99.84
2	20	101.27	99.71	100.85	100.11
3	30	101.65	101.96	98.48	98.55
4	40		100.48	100.69	99.85
5	50		100.22	101.09	100.71
6	60		100.33	98.99	98.96
7	70		101.50	101.28	100.72
8	80		100.00	98.22	102.02
Mean±SD		101.38±0.23	100.41±0.94	100.06±1.27	100.10±1.08

Table 3: Determination of NIC in Loxen® tablet by proposed methods and application of standard addition technique

D^1 method			D^2 method		DD^1 method		RD	
	Found%	Recovery %	Found%	Recovery%	Found%	Recovery%	Found%	Recovery%
Loxen® tablet labeled to contain 20 mg/tab. B. No. T0109 mean±SD	99.34±1.64	98.46 99.82 99.45 99.25 ±0.70	99.86±0.409	98.19 99.38 101.39 99.65 ±1.61	100.88±1.163	98.00 100.88 100.64 100.11±1.85	100.88±1.163	101.65 101.79 100.64 101.36 ±0.63

Table 4: Statistical comparison of the results obtained by the proposed methods and the reported method for determination of NIC hydrochloride

Values	Spectrophotometric method				Reported method[4]*
	D^1	D^2	DD^1	RD	
Mean percentage recovery	100.16	100.04	98.98	99.81	100.24
SD	1.24	0.99	1.42	1.77	1.26
variance	1.54	0.98	2.02	3.13	1.59
n	7.00	7.00	7.00	7.00	5.00
Student´s t-test (2.230)	0.10	0.30	1.58	0.46	
F-value	1.03 (4.53)	1.62 (4.53)	1.27 (6.16)	1.97 (6.16)	----------

CONCLUSION

The developed methods are simple, sensitive and rapid stability indicating ones for determination of NIC in pure form, in pharmaceutical formulations and in the presence of its alkaline induced degradation products. The RD method advantage over the derivative method that the signal to noise ratio is enhanced. These methods were simple, more convenient, less time consuming and economic stability indicating methods compared to chromatographic methods.

CONFLICT OF INTERESTS

Declare none

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