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Development and validation of a GC-FID method for determination of cocaine in illicit drug samples

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ABSTRACT: The objective of this study was to develop and validate a simple and reliable GC-FID method for quantitative determination of cocaine in illicit drug samples. Chromatographic conditions and detection parameters were optimized. Separation was performed on a HP-5 column (30 m-0.32 nm ID-0.25 µm) using an internal standard of n-tetracosan at the concentration of 0.25 mg/mL in methanol/chloroform (1:1) mixture. Validation of the method was performed by means of specificity, linearity, accuracy, precision, range, quantitation limit and detection limit. Method showed linearity with excellent correlation coefficients (r^2=0.9992) for cocaine. The limit of detection and limit of quantification values of GC-FID method for cocaine analysis were 1.80 µg /mL and 5.57 µg /mL, respectively while limit of linearity was 1200 µg /mL. Mean recovery value obtained from spike study was 101.20%, and relative error calculated after CRM analysis was equal to 1.0%, indicating that method was accurate. Inter-day stability of the instrument was proven by use of the control chart. The procedure described is relatively fast, simple, precise, and applicable for routine illicit drug analysis in forensic laboratories

KEYWORDS: Cocaine; forensic; toxicology; validation; GC-FID.

1. INTRODUCTION

Cocaine is a benzoid acid ester and an alkaloid found naturally in the coca plant, namely Erythroxylon coca or Erythroxylon novogranatense. Though cocaine was initially utilized as a local anesthetic [1, 2], however, it has become one of the most commonly abused illegal drugs in the world. Chemical structure of cocaine is shown in Figure 1.

Cocaine addiction is an unfavorable public health problem resulting with major economic, medical, and social damage [3]. Presently, cocaine is not involved in any prescription medications. Nonetheless, use of cocaine as a topical anesthetic is possible but restricted only in ear nose and throat surgery as well as ophthalmologic procedure or in skin suturing. Pharmacological effects of cocaine takes place when it inhibits reuptake of the neurotransmitters dopamine and norepinephrine, resulting with a rise in blood pressure, body temperature, and heart rate [4,5]. Before new psychoactive drugs (cathinones and synthetic cannabinoids) have appeared [6], cocaine was the second most problematic illicit drugs in Europe after cannabis according to 2010 Annual report on the state of the drugs problem in Europe [7]. Turkey is exposed both as a transit and destination country in accordance with cocaine trafficking which departs from Argentina, Brazil, Ecuador, Paraguay, and Venezuela. When Turkey is a target country of destination, cocaine is generally sent over West Africa. Consequently, cocaine can be transferred to inner locations by the roadways once it arrives to Europe and the Middle East. Furthermore, 1476 cocaine cases arose in Turkey in 2016. In those cases, 2201 suspects were arrested, and 845 kg of cocaine were seized [8].

In recent years, various chromatographic methods were developed to analyse cocaine content in both biological specimens and illegal drugs [9-12]. Thin-layer chromatography (TLC) emerges as a favorable alternative to immunoassays since it is one of simple and the most economical methods. However, low concentrations of drugs might be missed with such screening methods. Therefore, mass spectroscopy (MS)

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combined with gas chromatography (GC) and liquid chromatography (LC) are good examples for confirmation and quantification [13].

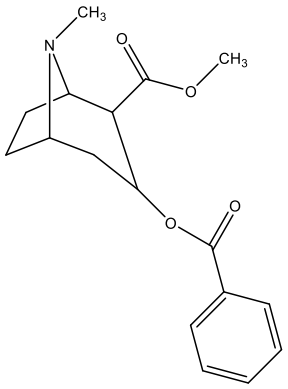

Figure 1. Chemical Structure of Cocaine

In recent years, various chromatographic methods were developed to analyse cocaine content in both biological specimens and illegal drugs [9-12]. Thin-layer chromatography (TLC) emerges as a favorable alternative to immunoassays since it is one of simple and the most economical methods. However, low concentrations of drugs might be missed with such screening methods. Therefore, mass spectroscopy (MS) combined with gas chromatography (GC) and liquid chromatography (LC) are good examples for confirmation and quantification [13]. The objective of this study is to develop and validate a GC-FID method for quantitative determination of cocaine in illicit drug samples. Though, there are many studies on the similar subjects, the present paper provided an improved chromatographic resolution and detection sensitivity during validation and optimization of the method, as the major significance and novelty of our paper. Last but not least, the study demonstrated an adequate separation of all analyte peaks within 13 minutes.

2. RESULTS and DISCUSSION

2.1. Optimization

To achieve the best performance from this chromatographic analysis, some important parameters were optimized. The major criteria included selection of the appropriate column, selection of concentration range in accordance with the cocaine concentration in illicit drug powders, evaluating the best oven temperature program, choice of a proper internal standard and establishing the linearity. From this point of view, primitive examinations were carried out for regulation and choice of the chromatographic conditions for the GC-FID analysis of cocaine. Since, the column plays an important role in an upgrated chromatographic separation [14], the choice of the convenient capillary column was considered on the basis of four important elements: stationary phase, column I.D., film thickness, and column length. According to Tony Taylor's 2015 paper, temperature influences retention and relative retention in GC. It may therefore not be unexpected, when temperature is changed, the selectivity of the separation is also altered [15]. In order to advance chromatographic resolution and detection sensitivity, the outcome of the oven temperature rate was investigated. For this purpose, not only various oven temperature programs but also ramp rates were examined up to highest oven temperature of 300 ^{o}C, and 280 ^{o}C was selected as the final temperature. After that, the gradient temperature program in the GC oven and the injection were investigated. The advanced program yields an adequate separation of all analyte peaks within 13 minutes. Using internal standard (IS) is recommended to prevent against possible miscalculations following injection of different sample volumes in the chromatographic equipment [13]. Therefore n-tetracosan was selected as IS. Choice of a convenient solvent is necessary to assure that both the internal standard and the cocaine sample are perfectly dissolved [2]. Hence, methanol/chloroform mixture (1:1, v/v) was selected as solvent.

An Agilent Model 6890N gas chromatograph was utilized during analyses. One mL of the prepared solutions was placed into an autosampler vial for analysis, and separation was achieved on a HP-5 column (30 m, 0.32 mm ID, 0.25 µm) using an IS (tetracosan at the concentration of 0.25 mg/mL) in methanol/chloroform (1:1, v/v) mixture. Ultrahigh purity (99.999 percent) hydrogen was chosen as the carrier gas with a flow rate of 1.5 mL/minute. The flame ionization detector and injection port were sustained at 280 °C. An Agilent 7683 Series Auto Injector was used during injection of samples. In the

splitless mode (20:1), 2 µL amounts of samples were injected. Then, an isothermally programmed oven tempreature was adjusted to 180 °C for 10.00 minutes, and nitrogen was utilized as the auxiliary make-up gas for the detector. Operating parameters of GC-FID system for cocaine analysis was given in Table 1.

Table 1. Operating Conditions for Cocaine Analysis by GC-FID

Column	30 m – 0.32 mm ID – 0.25 µm HP-5
Injection	Splitless: 1/20
Injector Temperature	285 °C
Carrier Gas	Hydrogen at 1.5 mL/min flow rate
Oven Temperature Ramp Program	Initial Temperature :180 °C Start Time :1 minute Temperature Rate :10 °C/min Final Temperature :280 °C or 275 °C Final Time :10 minutes
Detector Temperature	275°C
Analysis Time	13 minutes

2.2. Method Validation

Validation of the method was performed according to International Conference on Harmonization (ICH) guidelines [16] by means of specificity, linearity, accuracy, precision, range, quantitation limit and detection limit.

2.2.1. Specificity

Specificity of an analytical method can be defined as the detection ability of the desired analyte in the existance of other components that can be expected to be present, such as impurities, degradation products, and matrix components [17-19]. To assess the specificity of this chromatografic method, our working solution: methanol/chloroform (1:1, v/v) mixture and placebo solution containing the IS (tetracosan at the concentration of 0.25 mg/mL) without the cocaine were injected into the GC-FID system. The specificity of the method was performed in presence of our working solution and placebo, which has cocaine free solution. A representative chromatogram of cocaine and paracetamol standard solution is shown in Figure 2(A). As can be seen in Figure 2(B), there is no peak associated to placebo or dilution solution was detected at the retention time of cocaine. Figure 2(C) demonstrates the sample chromatogram.

2.2.2. Accuracy

Accuracy can be defined as the closeness of a measured value to an accepted reference value or known value. The accuracy of the method was asssessed by means of the percentage recovery data from spike analysis. According to our experience, cocaine samples seized in Turkey generally contain paracetamol. Reference paracetamol and cocaine solution were therefore spiked in three different amounts. The mean of recovery was found as 101.20% for cocaine (see Table 2). In addition, relative error (RE) and coefficient of variation (CV) were also used to assess the accuracy of the method. For this purpose, 0.25 mg of n-tetracosan was dissolved in 1.0 mL of standard solution (1.0 mg/mL of cocaine), and the final solution was analyzed 11 times. The results were compared to the certified values for accuracy and precision of the method. A good agreement was obtained among certified value (1.00±0.00 mg/mL) and measured concentration (1.010±0.003 mg/mL). CV and RE values were found as 0.29% and 1.0%, respectively, indicating that the method was accurate (see Table 3).

2.2.3. Precision

The precision of method was evaluated in terms of repeatability, intermediate precision and reproducibility parameters. The reproducibility of the proposed method was determined by analysis of six different samples at the same concentration of 400 µg/mL from the same certified reference solution of cocaine. Paracetamol concentration of these samples were adjusted as 200 µg/mL to make sure the matrix effect. The mean of measured cocaine concentrations was found as 399.2±5.3 µg/mL with 1.33% relative standard deviation (RSD). The result of reproducibility study was shown in Table 4. Repeatability was controled by injecting six individual sample of cocaine at 300 µg/mL concentration while the intermediate precision was assessed by two analysts. Mean cocaine concentrations from Analyst-A and Analyst-B were

calculated as 305±2.0 µg/mL and 304±3.0 µg/mL, respectively. Repeatability study was summarized in Table 5.

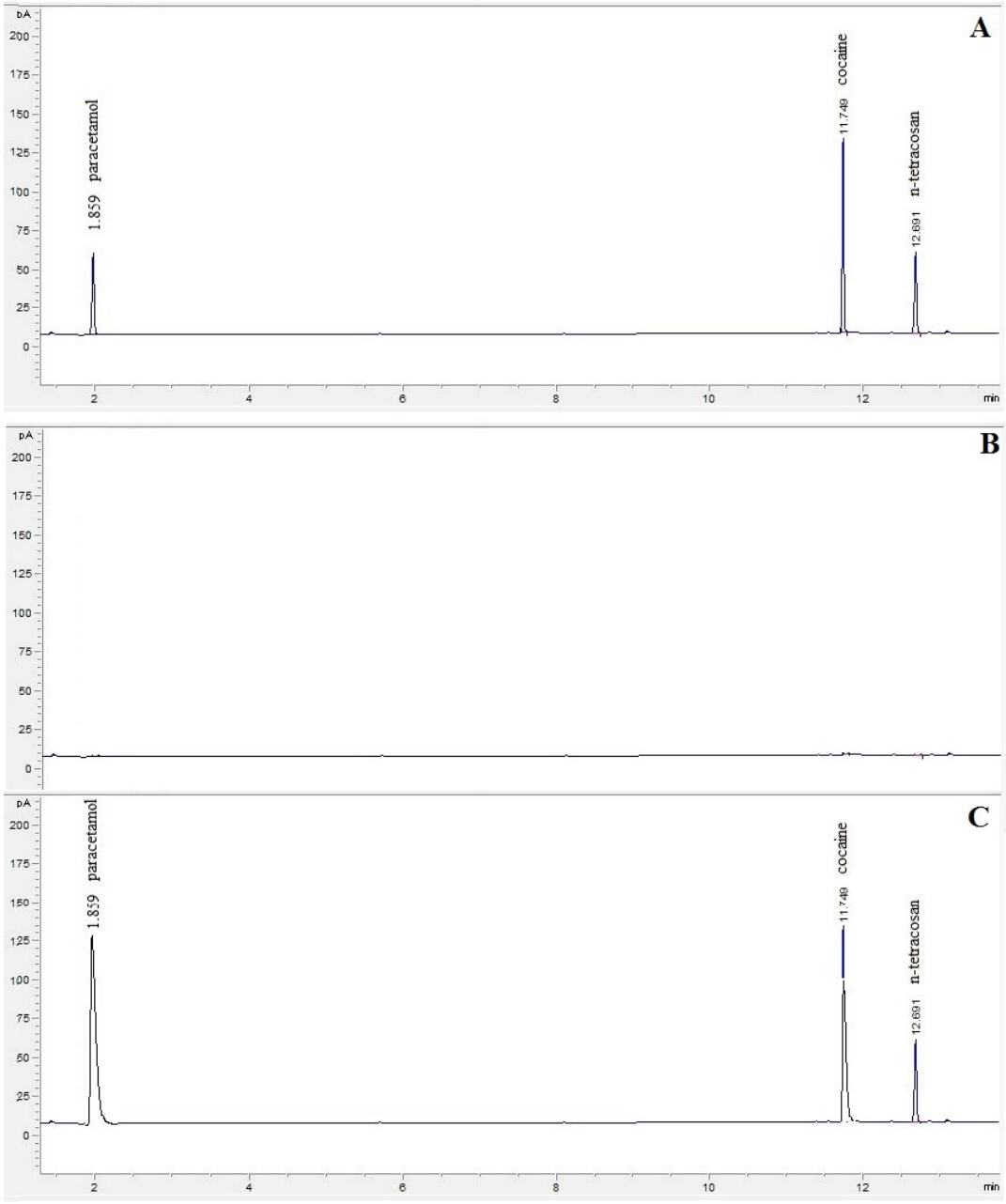

Figure 2. The chromatograph of (A) cocaine and paracetamol standard injection, (B) placebo and working solution, (C) sample chromatogram, obtained after the analysis according to proposed GC-FID method.

Table 2. Data of Recovery Study for Cocaine Analysis by GC-FID (Before the analysis, 0.25 mg of n-tetracosan was dissolved in 1 mL amount of the cocaine and paracetamol mixture. Certified concentrations for cocaine and paracetamol solutions were individually equal to 1.00±0.00 mg/mL before spiking them up at three different amounts as stated in the Table 2.)

Spiked Paracetamol (µL)	Spiked Cocaine (µL)	Theoretical Cocaine Concentration (µg/mL)	Measured Cocaine Concentration (µg/mL)	Recovery (% R)
300	700	700	694±5.0	99.1
450	550	550	562±6.0	102.2
700	300	300	307±7.0	102.3
Mean Recovery (% R)				101.2

2.2.4. Control Chart

The control chart study allowes for tracking inter-day and intra-day variation in peak intensity [20]. An appropriate procedure for monitoring inter-day stability of the instrument was proven by use of the control chart. A mixture solution containing cocaine at 600 µg/mL and paracetamol at 200 µg/mL concentration was analyzed for 22 times by GC-FID method for 11 days, and the mean concentration of cocaine was found as 571.0±21.0 µg/mL. After that, warning limits were calculated from following formula: Warning Limits = xmean±2σ. Lowest warning limit (LWL) and upper warning limit (UWL) were equal to 557.8 µg/mL and 585.0 µg/mL, respectively. Similarly, control limits were calculated from the formula: Control Limits = xmean± 3 σ. Lowest control limit (LCL) and upper control limit (UCL) were equal to 550.1 µg/mL and 591.7 µg/mL, respectively. The control chart study was demonstrated in Figure 3.

3. CONCLUSION

Addiction to cocaine is a well-known global health problem. Turkey is also affected from international illegal cocaine traffiking both as a transit and destination country. In this study, GC-FID method for cocaine analysis in illicit drug samples was developed and validated for accuracy, precision and linearity. The mean recoveries obtained from CRMs analysis were found as 101.20% with relative error equal to 1.0%, which revealed the method was accurate. The method provided LOD and LOQ equal to 1.8 µg/mL and 5.57 µg/mL, respectively. The GC-FID method is relatively fast, simple, precise, and applicable for routine forensic and pharmaceutical analysis.

Table 3. Assessment of Relative Error (RE) and Coefficient of Variation (CV). Before the analysis, 0.25 mg of n-tetracosan was dissolved in 1 mL amount of the cocaine standard solution.

Standard Solution	Number of Analysis (n)	Certified Value (mg/L)	Measured Value (mg/L)	RE (%)	CV (%)
Cocaine standard at the concentration of 1 mg/mL (Sigma-Aldrich)	11	1.000±0.000	1.010±0.003	1.000	0.290

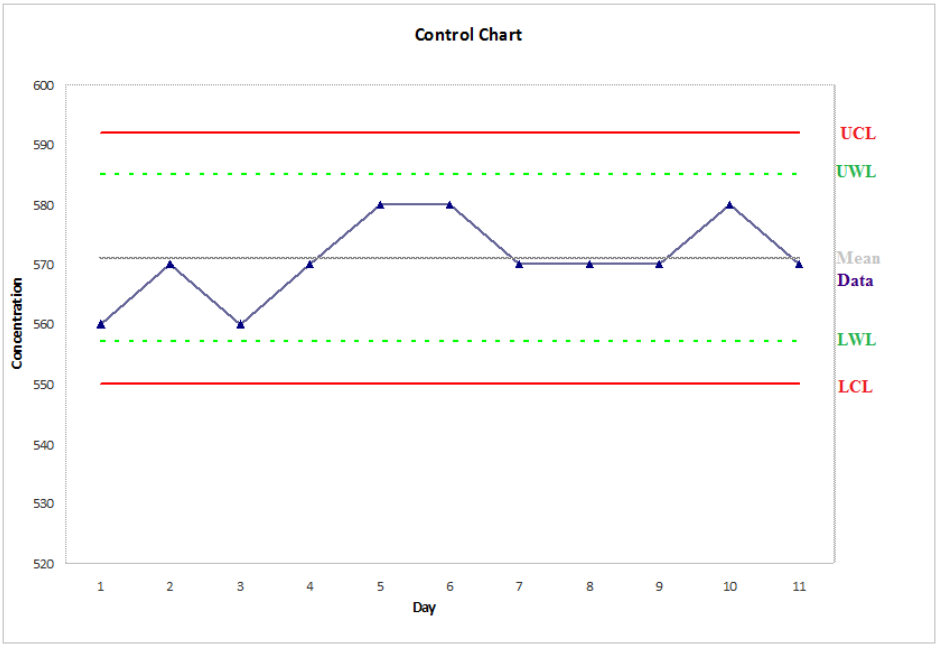

Figure 3. Control chart of cocaine, performed by GC-FID. Concentration of cocaine is given in µg/mL while UCL, UWL, LWL and LCL stand for upper control limit, upper warning limit, lowest warning limit and lowest control limit, respectively. Blue line represents the stability in the cocaine concentrations measured over days.

4. MATERIALS AND METHODS

4.1. Instrumentation

The analysis was performed using Agilent GC 6890N (Santa Clara, California, USA) equipped with a flame ionization detector (FID) and an automated liquid sampler. An Agilent 7683 Series Auto Injector was utilized for injection of samples. This instrumentation was utilized for validation and optimization of an analytical method based on determination of cocaine in illicit samples.

Table 4. The results demonstrating the reproducibility of the method for precision study.

Sample	Measured Cocaine Concentration (µg/mL)
1	394
2	392
3	404
4	402
5	405
6	**398**
Statistics	
Mean±SD [a]	399.2±5.3
RSD% [b]	1.33

[a] SD: Standard deviation, [b] RSD: relative standard deviation

4.2. Standard Solutions and Reagents

Stock solutions of methanol and chloroform were obtained from Merck (Darmstadt, Germany). Also, n-Tetracosan powder was purchased from Merck (Darmstadt, Germany). Certified reference material (CRM) of cocaine and paracetamol solutions and powders were obtained from Lipomed Services to Health, Switzerland. All the other chemicals and solvents used during laboratory work were of analytical reagent grade. Ultrapure water (Merck Millipore Direct-Q8, Germany) with a resistivity of 18MΩ.cm, was used to prepare the solutions during the experimental process.

Table 5. The results illustrating the repeatability and intermediate precision. Six individual cocaine samples at the concentration of 300 µg/mL were analyzed by two analysts

Cocaine Sample	Analyst-A (µg/mL)	Analyst-B (µg/mL)
1	308	301
2	302	308
3	305	302
4	304	306
5	306	303
6	303	304
Statistics		
Mean±SD	305±2.0	304±3.0
%RSD	0.66	0.99

4.3. Sample Preparation and Procedure

All samples and calibration standard solutions were prepared by use of appropriate amount of our working solution: methanol/chloroform (1:1, v/v) mixture containing the IS (tetracosan at the concentration of 250 µg/mL). In order to prepare the calibration standards at the concentrations of 0, 150, 300, 600, 900 and 1200 µg/mL, cocaine powder standards were diluted in our working solution described above. The solution was stored at 4±1°C when not in use and warmed to room temperature before use. All calibration standards were analyzed 5 times, and a calibration curve of area cocaine/area tetracosan versus concentration of

cocaine standards was drawn (Figure 4). The correlation coefficient (r) and equation of the calibration curve for cocaine were respectively found to be r=0.9995 and y=2.7127x where y stands for area cocaine/area tetracosan, and x is the cocaine concentration in mg/mL. The chromatogram of cocaine standard at 600 µg/mL, obtained after analysis according to proposed GC-FID method, was illustrated in Figure 2.

4.4. Limit of Detection, Quantification and Linearity

The limit of detection (LOD) and lowest limit of quantification (LOQ) were determined based on the standard deviation of the response and the slope of the calibration curve, according to International Conference on Harmonization (ICH) guidelines [16], LOD=3.3σ/S, LOQ=10σ/S, where σ is the standard deviation of the response and S is the slope of the calibration curve. The LOD and LOQ values of GC-FID method for cocaine analysis were 1.80 µg /mL and 5.57 µg /mL, respectively. Limit of linearity (LOL) is the concentration at which the calibration curve departs from the linearity. Dynamic range refers to concentration intervals from LOQ to LOL, which was found between 5.57 µg/mL and 1200 µg/mL, in this study.

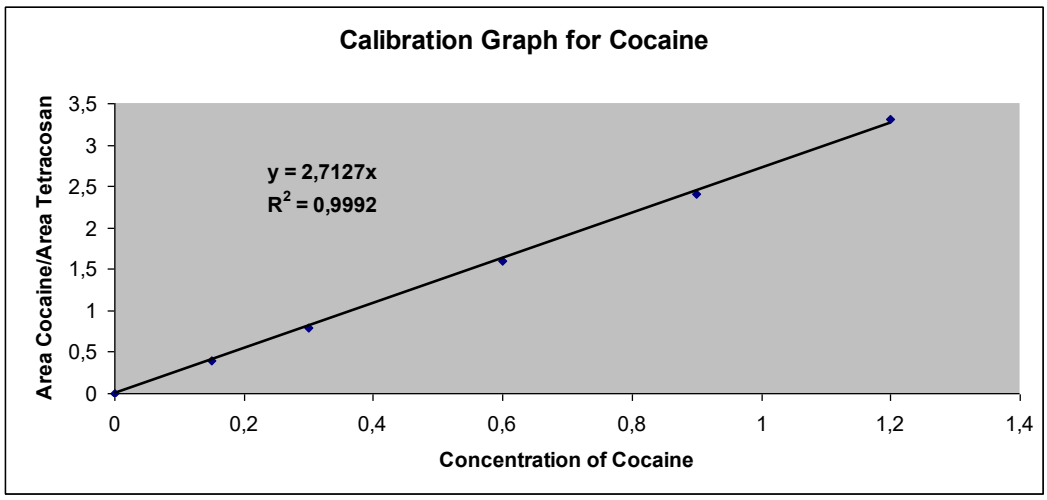

Figure 4. Calibration graph of cocaine, constructed after GC-FID analyses. Concentration of cocaine is given in mg/mL.

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