International Journal of Academic Scientific Research
ISSN: 2272-6446 Volume 3, Issue 4 (November-December 2015), PP 106-114
www.ijasrjournal.org

Sensitive spectrophotometric methods for Determination of zolmitriptan in bulk form and in tablets via complex formation with tow sulphonphthalein acid dyes

Amir Alhaj Sakur, Reem Obaydo
(Department of Analytical and food Chemistry, Faculty of Pharmacy/ University of Aleppo, Syria)

ABSTRACT *Two simple, sensitive and extraction-free spectrophotometric methods have been developed for the determination of zolmitriptan (ZMT) in pure form and in pharmaceutical formulations. The methods are based on the formation of yellow coloured ion-pair complexes between ZMT and two sulphonphthalein acid dyes, bromocresol green (BCG) method (A) and bromocresol purple (BCP) method (B) with absorption maximum at 411 nm and 403 nm for BCG and BCP, respectively. The stoichiometry of the complex in either case was found to be 1: 1. Reaction conditions were optimized to obtain the maximum colour intensity. Beer's law was obeyed in the concentration ranges of 0.5–15.0and 0.375–12.0 µg/mL with BCG and BCP, respectively. The limits of quantification (LOQ) were 0.212 and 0.144 µg/mL for BCG and BCP methods, respectively . molar absorptivity (Ɛ) values were 24795, and 34548 L/moL.cm for BCG and BCP methods, respectively.The proposed methods have been applied successfully to the analysis of ZMT in pure form and in its dosage forms and no interference was observed from common excipients present in pharmaceutical formulations. Statistical comparison of the results with the reference method showed excellent agreement and indicated no significant difference in accuracy and precision.*

Keywords: *Ion-pair complexes, Spectrophotometry , Sulphonphthalein acid dyes, Zolmitriptan.*

1. INTRODUCTION

Zolmitriptan (ZMT), 4(S)-4-[3-(2-dimethyl aminoethyl)-1H- 5-indolyl-methyl]-1, 3-oxazolan-2 one (Figure 1) belongs to a group of medicines known as Serotonin 5-HT1D Receptor Agonists. It works by stimulating serotonin receptors in the brain. Serotonin is a natural substance in the brain that, among other things, causes blood vessels in the brain to narrow. It is used to treat severe migraine headaches [1].

The assay of ZMT in pure and dosage forms, as far as we know, is not official in any pharmacopoeia, and therefore, requires much more investigation. The different analytical methods that have been reported for its determination include HPLC with mass spectrometry detection [2-7], with coulometric detection [8], electrospray ionization mass spectrometry [9], tandem mass spectrometry [10], fluorescence detection [11,12] in pharmaceutical preparations and biological fluids and spectrophotometric and flourometric methods [13-20]. In the present work, spectroscopic analytical study for the analysis of zolmitriptan in pure and its Syrian pharmaceutical dosage forms through complexation with Sulphonphthalein dyes in dichloromethan medium has been applied.

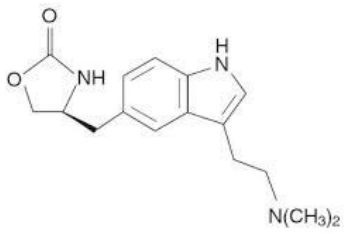

Fig. 1 Zolmitriptan structure.

2. EXPERIMENTAL

2.1. Apparatus

spectrophotometric measurements were made in Jasco company (Japan) model V650. UV-Visible spectrophotometer with 1.00 cm quartz cells. Ultrasonic processor model powersonic 405 was used to sonicate the sample solutions. The diluter pipette model DIP-1 (Shimadzu), having 100 µL sample syringe and five continuously adjustable pipettes covering a volume range from 20 to 5000 µL (model PIPTMAN P, GILSON), were used for preparation of the experimental solutions.

2.2. Reagents and solutions

Pharmaceutical grade zolmitriptan (ZMT 99.88%) was received from (Razi pharmaceutical industry) as a gift. A stock solutions of ZMT (5.0×10^{-3} M) were prepared by dissolving the appropriate weight of ZMT in 25 mL chloroform for method A and in dimethylsulfoxide for method B and the volume was diluted to the mark in a 250 mL calibrated flask with dichloromethane for both methods. Working standard solutions were prepared from suitable dilution of the standard stock solution. All solutions are stable for a period of 3.0 days when refrigerated (4°C).

Working standards were prepared daily by added different volumes of stock solutions to 1.5 mL of reagent BCG (5.10^{-4}M) and 1.5 ml of reagent BCP ($1.\ 10^{-3}$ M) diluting to 10 mL with dichloromethane.
The concentration of ZMT (0.375, 0.5, 1. 5, 3.00, 6.00, 9.00, 13.00,15.00, µg.mL^{-1}). Were used for the analysis of ZMT by the spectrophotometric methods (A and B) after 5 min. The methods were based on the formation of an ion-pair complex between Sulphonphthalein dyes (BCG,BCP) and ZMT in dichloromethane medium. The colored product was quantified spectrophotometrically using absorption bands at 411 nm for complex of (ZMT–BCG) and at 403 nm for (ZMT–BCP).

Bromocresol green, BCG (1.10^{-3}M) Prepared by shaking 70 mg of BCG dye (BDH ,ENGLAND COMPANY) in 10 mL dichloromethane to dissolve and made up to mark with dichloromethane in a 100 mL calibrated flask.

Bromocresol purpur ,BCP (1.10^{-3}M) Prepared by shaking 55.64 mg of BCP dye (Merck) in 10 mL dichloromethane to dissolve and made up to mark with dichloromethane in a 100 mL calibrated flask.

Zolmitriptan tablets ,Zomigrain (National Company for pharmaceutical industry, Aleppo, Syria) containing 5 mg and 2.5mg Zoloraz (Razi pharmaceutical industry , Aleppo, Syria) containing 5 mg and 2.5 mg ,Zomitan (pharmasyr Company, Damascus, Syria) containing 5 mg and 2.5mg from local medical stores.

All reagents and solvents were of analytical grade.

2.3. Spectrophotometric procedure

Increasing volumes of ZMT working standard solution were transferred into series of 10 mL volumetric flasks that contain 1.5 mL of BCG reagent (1.10^{-3}M) for method A and 1.5 ml of BCP (1.10^{-3}M) for method B. Solutions were mixed gently and allowed to stand at room temperature for 5 minutes. Volumes were made up to mark with dichloromethane and mixed before the spectra was recorded at 411 nm for method (A) and,403 nm for method (B) against reagent blank that had been treated similarly.

2.4.Determination of ZMT/Dye stoichiometric relationship

The composition ratio of drug ZMT to dyes (BCG-BCP) of the colored complex was determined using the molar ratio and continuous variation methods[20].

2.5. Procedure for pharmaceutical samples

Ten individual tablets were weighed and pulverized carefully. An accurately weighed amount of the powder equivalent to 5 mg of ZMT was transferred into 25 mL volumetric flask and dissolved in 20 mL of

chloroform for method (A) and in dimethylsulfoxide for method (B) . The content of the flask was sonicated for 20 min then diluted to volume with chloroform for method (A) and with dimethylsulfoxide for method (B). A portion of this solution was centrifuged at 5000 rpm for 10 minutes. 2.5 mL of the supernatant was then transferred into 10 mL volumetric flask, and diluted with dichloromethane up to mark. Then suitable volume was transferred into 10 mL volumetric flask and procedure was continued to used for the analysis of ZMT by the spectrophotometric method after 5 min.

3. RESULTS AND DISCUSSION

3.1.Optimization of reaction conditions

3.1.1.Effect of reaction time and stability

The optimum reaction time for the development of color at ambient temperature (25±2C°) was studied and it was found that a 5 min standing time was sufficient for the complete formation of ion–pair complexes in A and B methods. The formed color was stable for more than 24 h in methods A and B.

3.1.2.Solvent effect

In order to select a suitable solvent for preparation of the reagent solutions used in the study, the reagents were prepared separately in different solvents such as, chloroform methanol, dichloroethane and dichloromethane, and the reaction of ZMT with BCG or BCP was followed. In methods A and B, dichloromethane was best suited for the preparation of BCG and BCP solutions, respectively. The chloroform solvent was found to be the ideal solvent for preparation of ZMT for method A and the dimethylsulfoxide solvent was found to be the ideal solvent for preparation of ZMT for method B .Similarly, the effect of the diluting solvent was studied for all methods and the results showed that none of the solvents except dichloromethane formed sensitive and stable colored species in methods (A and B). Therefore, dichloromethane was used for dilution throughout the investigation. dichloromethane was preferred as the most suitable solvent because in this medium, the reagent blank gave negligible blank absorbance and the formed ion-pair complex was found to exhibit higher sensitivity and stability. In other solvents, the reagent blank yielded high absorbance values.

3.1.3. Effect of dye concentration

The influence of the concentration of BCG and BCP on the intensity of the color developed at the selected wavelength and constant drug concentration was studied. As shown in Figure 2 the constant absorbance readings were obtaind between(0.4-3.0 mL) of (1.10^{-3}M) of BCG in method A and (0.2-3.0 mL) of (1.10^{-3}M) BCP in method B. Hence, 1.5 mL of each BCG and BCP was used for methods A and B, respectively.

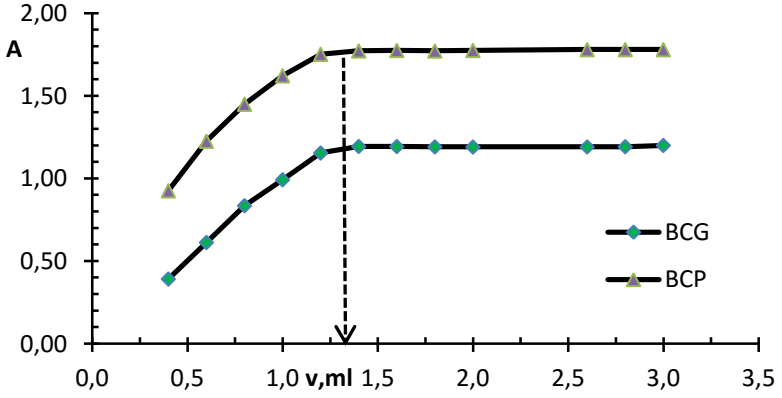

Fig.2. Effect of the volume added of dye (BCG-BCP) solution on the absorbance of ZMT-Dye complex

3.1.4.Stoichiometric ratio

Molar ratio method**:** The stoichiometry of (ZMT:Dye) complex by molar ratio method according to following equation: A_{max}= f([ZMT]/[Dye], confirms that the ratio of complex ZMT:BCG and ZMT:BCP are equal to 1:1 for both methods (Figure 3).

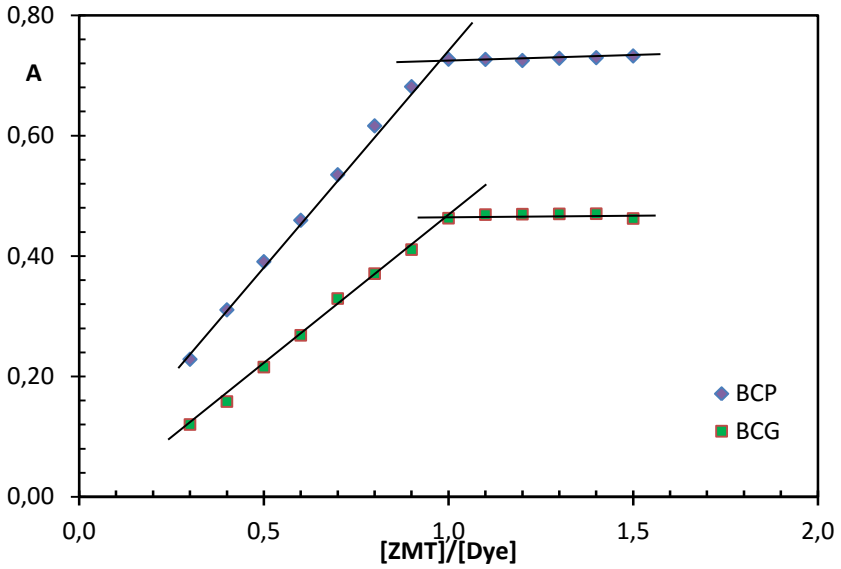

Fig.3 Molar ratio plots for(ZMT–Dye) complex.

Job's method : In order to establish the stoichiometry of ZMT and dyes (BCG,BCP) complex by Job's method of continuous variations was applied. In both cases, the plot reached a maximum value at a mole fraction of 0.5 which indicated the formation of 1:1 (ZMT:Dye) complex (Figure 4) between ZMT and BCG or BCP .

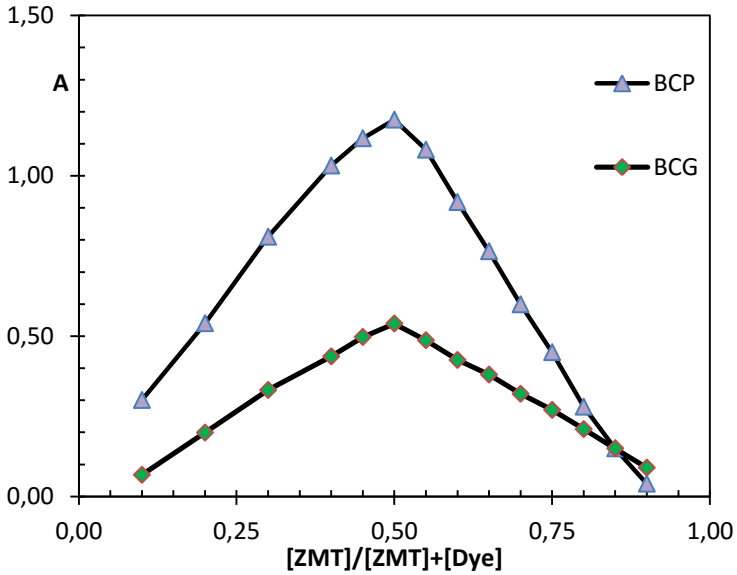

Fig.4 Continuous variations plots for (ZMT-Dye) complex.

3.2.Validation of the proposed method

3.2.1 Linearity

Under the optimum experimental conditions, standard calibration curve was constructed at eight concentration for method A and seven concentrations for method B levels (n=5) (figure8). The correlation coefficient was 0.9999 for method A ,and 0.9998 for method B indicating very good linearity, over the concentration range of 0.5 – 15.0 μg/mL for method A and 0.375-12 μg/mL for method B . The intercept, slope, limit of detection (LOD), and limit of quantitation (LOQ) are summarized in Table 1. LOD and LOQ values were calculated as $3.3S_b$/m and $10S_b$/m, respectively. Where molar absorptivity of regression and Sandell sensitivity [20, 21] Table 1.

Table 1. Statistics and analytical parameters of ZMT determination by BCG method (A) and by BCP method (B).

Parameter	Result(A)	Result (B)
λ_{max} (nm)	411	403
Linear range (μg/mL)	0. 5–15.0	0.375 – 12.0
Slope	0.0839	0.1279
Molar absorptivity (ε), L/ moL.cm	24795	34548
Intercept	0.0011	0.0105
Sandell sensitivity, μg/ cm^2	0.013	0.0082
Correlation coefficient	0.9999	0.9998
Limit of detection (μg/mL)	0.0627	0.0495
Limit of quantification (μg/mL)	0.19	0.15

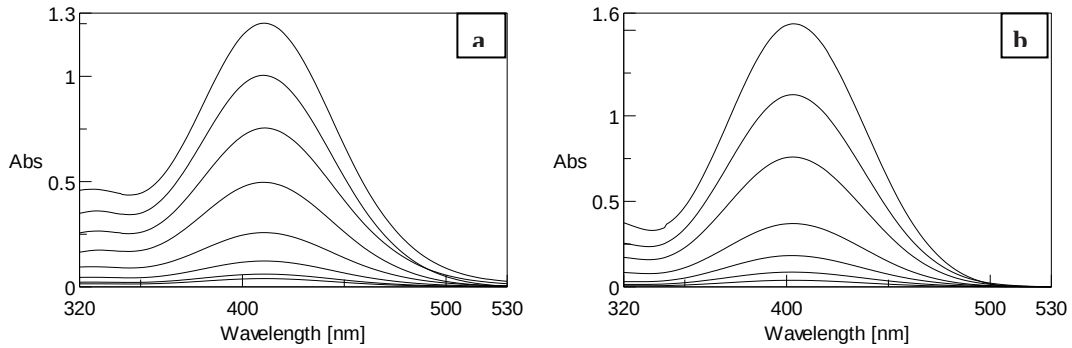

Figure 8: UV-Vis spectra of ZMT complex with (a) BCG method A,(b) BCP method B

3.2.2.Selectivity

Zolmitriptan was determined in the presence of possible excipients and additives such as lactose, microcrystalline cellulose, sodium starch glycolate and magnesium stearate. Under the experimental conditions employed, to a known amount of zolmitriptan (50 μg/mL), excipients in different concentrations were added and studied. Results of the % recovery are presented in Table 2. Excipients up to the concentrations shown in Table 2 do not interfere with the assay in both methods . In addition, recoveries in most cases were around 100% and the lower values of the RSD indicate the good precision of the method. The potential impurities such as (4*R*)-4-[[3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl]methyl]- 2-oxazolidinone and (4*S*)-4-(4-aminobenzyl)-2-oxazolidinone do not contain the tertiary amines and may be present at trace levels in pharmaceutical formulations and in bulk drugs. The proposed methods (A and B) are based on ion pair reaction between the

tertiary amino group of zolmitriptan and Sulphonphthalein acid dyes.[16] Thus these impurities will not cause interference in the determination of zolmitriptan.

Table 2. Percent recovery of ZMT in the presence of excipients 50 µg/mL of ZMT was taken for interference studies

Excipient	Taken (µg/ mL)	Recovery % of ZMT (RSD %) (n = 5)	
		Method A (BCG)	Method B (BCP)
Lactose	500	99.7 (0.37)	99.8 (0.33)
Microcrystalline cellulose	400	99.8 (0.28)	99.5 (0.23)
Sodium starch glycolate	100	99.2 (0.26)	99.3 (0.27)
Magnesium stearate	50	100.1 (0.36)	99.9 (0.29)

3.2.3 Precision

The repeatability of proposed methods were estimated by measuring five replicate samples of each concentration of zolmitriptan prepared in one laboratory on the same day .The precision expressed as the relative standard deviation (RSD%) ranged from 0.66% to 3.44% (method A) ,and from 0.89% to 3.36% (method B) for the smallest concentration, indicating good precision Table 3.

Table 3. Precision for determination of ZMT in pure form using proposed methods(A,B)

Method	Taken ZMT (µg/mL)	[a]Found ZMT (µg/mL)	SD (µg/mL)	RSD%	Recovery %
BCG (A)	0.500	0.4899±0.0209	0.0169	3.44	97.97
	0.750	0.7402±0.0277	0.0223	3.01	98.69
	1.500	1.5042±0.0236	0.0190	1.27	100.28
	3.000	2.9976±0.0426	0.0343	1.15	99.92
	6.000	5.9965±0.0710	0.0572	0.95	99.94
	9.000	9.0887±0.0984	0.0792	0.87	100.99
	12.00	11.9267±0.1271	0.1024	0.86	99.39
	15.00	15.0072±0.1238	0.0997	0.66	100.05
BCP (B)	0.375	0.3788±0.0158	0.0127	3.36	101.02
	0.750	0.7532±0.0312	0.0251	3.34	100.43
	1.500	1.5113±0.0473	0.0381	2.52	100.75
	3.000	3.0046±0.0837	0.0674	2.24	100.15
	6.000	6.0256±0.1418	0.1143	1.90	100.43
	9.000	8.8658±0.1042	0.0839	0.95	98.51
	12.00	12.0874±0.1333	0.1074	0.89	100.73

[a] Average of five determination ± Confidence limit

3.2.4.Intraday and inter day results**:**

The accuracy and precision of the methods were evaluated by performing five replicate analysis in pure drug solution at four different concentration levels (within the working range). Percentage relative standard deviation (RSD %) as precision and percentage relative error (RE %) as accuracy of the proposed spectrophotometric (A,B) methods were calculated. The relative standard deviation (RSD) values were less than 2% in all cases, indicating good repeatability of the suggested methods.

The percentage relative error calculated using the following equation:

RE % = [(found – taken) / taken]×100

The intra-day and inter-day precision and accuracy results show that the proposed methods have good repeatability and reproducibility Table 4.

Table 4 . precision and accuracy results

Method	Taken ZMT µg/ml	Intra-day n=5			Inter day n=5		
		[a]Found ZMT±CL µg/ml	[b]RSD%	Recovery%	[a]Found ZMT±CL µg/ml	[b]RSD%	Recovery%
BCG (A)	2	1.988±0.089	0.47	99.40	1.980±0.026	0.54	99.20
	4	3.980±0.028	0.68	99.50	3.996±0.0377	0.90	99.90
	8	7.984±0.87	1.04	99.80	7.944±0.046	0.55	99.30
	12	11.904±0.11	0.89	99.20	11.892±0.089	0.71	99.10
BCP (B)	2	1.998±0.186	0.53	99.90	1.984±0.013	0.61	99.20
	4	3.972±0.05	1.20	99.30	3.988±0.043	1.03	99.70
	6	5.946±0.047	0.76	99.10	5.946±0.054	0.87	99.10
	8	7.916±0.12	1.46	98.95	8.032±0.114	1.35	100.40

[a] Mean ± Confidence limit, [b] Mean of five determination

3.3.5. Accuracy

Accuracy is judged by comparing the results obtained from the presently proposed method, that has been applied on commercial tablets, with those obtained from a reference method such as HPLC .The resulted values were statistically compared with each other Table 5 using *t*- and *F*-tests. With respect to *t*- and *F*-tests, no significant differences were found between the calculated values of both the proposed and the reported methods at 95% confidence level.

Table 5. determination of ZMT in tablets

Brand name	Label claim	[a]Average ZMT found ± SD [b](Recovery%)			*t*- *(F-)* [c]test	
		Reference method[8]	Proposed method			
			Method A	Method B	Method A	Method B
Zomitan	2.5 mg/tab	2.48±0.122 (99.20)	2.49±0.148 (99.55)	2.51±0.124 (100.42)	0.15(1.47)	0.54(1.03)
Zomitan	5 mg/tab	5.02±0.106 (100.40)	4.99±0.146 (99.88)	5.08±0.112 (100.40)	0.46(1.89)	1.19(1.17)
Zomigrain	2.5 mg/tab	2.49±0.102 (99.60)	2.51±0.104 (100.33)	2.52±0.196 (100.93)	0.43(1.03)	0.34(3.69)
Zomigrain	5 mg/tab	5.04±0.121 (100.80)	5.10±0.123 (102.06)	5.07±0.126 (101.38)	1.09(1.03)	0.53(1.08)
Zoloraz	2.5 mg/tab	2.51±0.111 (100.45)	2.55±0.132 (101.96)	2.60±0.156 (103.90)	0.67(1.41)	1.29(1.97)
Zoloraz	5 mg/tab	5.03±0.107 (100.60)	4.96±0.215 (99.15)	5.05±0.204 (100.91)	0.73(4.03)	0.22(3.63)

[a]Average and standard deviation of five determinations for the proposed method
[b] Recoveries were calculated considering the labeled amount reported by the manufacturer.
[c] the tabulated *t* value at 95% confidence limit for 4 degrees of freedom (n =5) is 2.776 and the tabulated *F* value at 95% confidence limit for 4 degrees of freedom for the proposed methods is 6.25.

3.3.Application to Tablets

The proposed methods were applied to the determination of ZMT in tablets. The results in Table 5 showed that the methods are successful for the determination of ZMT and that the excipients in the dosage forms do not interfere. A statistical comparison of the results for determination of ZMT from the same batch of

material by the proposed and reference method is shown in Table 5.The results agreed well with the label claim and also are in agreement with the results obtained by the reference method .Statistical analysis of the results using Student's t-test for accuracy and F-test for precision revealed no significant difference between the proposed and reference method at the 95 % confidence level with respect to accuracy and precision Table 5.

4. CONCLUSION

This paper describes application of extraction-free ion–pair complex formation for the quantification of ZMT in pharmaceutical formulations. Compared with the existing visible spectrophotometric methods, the proposed methods are simple, selective, cost-effective, free from auxiliary reagents and more sensitive as can be seen from the molar absorptivity values. Moreover, the proposed methods are free from tedious experimental steps such as heating or extraction step .

The most attractive feature of these methods is its relative freedom from interference by the usual tablet diluents and excipients in amounts far in excess of their normal occurrence in pharmaceutical formulations. The statistical parameters and the recovery data reveal good accuracy and precision of the methods. Hence, recommended methods are well suited for the assay and evaluation of drug in quality control laboratories.

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