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Research Article

**Comparative evaluation of quercetin content in three
varieties of *Allium cepa* using TLC densitometry**

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ABSTRACT

Introduction: Herbal medicines are known for their multiple compounds which are responsible for their bioactivity. This creates a challenge in establishing quality control standards for raw materials and the standardization of finished herbal drugs. Currently, there is a common practice of selecting one or more compounds as markers for purposes of identification and quality assessment. In the present study a TLC densitometric method for determining the quantity of quercetin in extracts of outer scales of three different commercial varieties of *Allium cepa* (onion) was validated according to the ICH guidelines with respect to linearity, sensitivity, precision and accuracy.

Materials and Methods: The test samples of all three varieties of *A. cepa* were prepared with methanol (90%). Similarly, the flavonoid rich fractions of each variety were prepared by fractionation with ethyl acetate. The methanol (90%) extract and flavonoid rich fraction of each variety were subjected to standardization using TLC densitometric analysis using quercetin as a marker compound.

Results: The TLC densitometric studies show that the quercetin content was found to maximum in L28 variety of onion.

Conclusion: This is a simple and precise method for evaluating the quality of onion extracts. This may be used in future to determine the quantity of the marker – quercetin, and help in standardization of *A. cepa* extracts.

Keywords: *Allium cepa*, different varieties, quercetin, TLC-densitometry

INTRODUCTION

Herbal drugs, singly and in combination, contain a myriad of compounds in complex matrices in which no single active constituent is responsible for the overall efficacy. This creates a challenge in establishing quality control standards for raw materials and the standardization of finished herbal drugs. Currently, there is a common practice of selecting one or more compounds as markers for purposes of identification and quality assessment¹. In recent years, standardization of herbal products through marker profiling of various active ingredients is a convenient and effective option for the quality control of herbal products, especially when there is lack of pure and authentic standards for the identification of the active ingredients of these complex natural products^{2,3}. Planar chromatography,

and its high-performance version (HPTLC), coupled with densitometric detection, is among the various methods reported for the quality control of pharmaceutical products⁴. HPTLC nowadays has become a powerful analytical technique because of its reliability, simplicity and speed. HPTLC is becoming the routine analytical technique due to its advantages of low operating cost, high sample throughput, need for minimum sample clean-up and for its high accuracy, precision and reproducibility^{5,6}.

HPTLC also facilitates repeated detection (scanning) of the chromatogram with same or different parameters. Simultaneous assay of several components in a multicomponent formulation is possible⁷. The aim of the present work is to develop

and validate an accurate, specific and reproducible HPTLC method for determination of marker compound in different varieties of a plant.

Onion (*Allium cepa*) a common food plant is a rich source of several phytonutrients is also used in the treatment and prevention of a number of diseases including cancer⁸, coronary heart disease⁹, obesity, hypercholesterolemia^{10,11}, diabetes type 2^{12,13}, cataract¹⁴, neuroprotective¹⁵, antimicrobial^{16,17} and disturbances of the gastrointestinal tract (e.g. colic pain, flatulent colic and dyspepsia). Most of these activities are related to the flavonoids, thiosulfates and sterols. Onion is the one of the richest sources of flavonoids and organosulphur compounds. Onion possesses a high level of anti-oxidant activity¹⁶, which is attributed to the flavonoids quercetin, kaempferol, myricetin and catechin; pigments such as anthocyanins found in red onions¹⁸. In present study various extracts of outer scales of three Indian varieties of *Allium cepa* i.e. Agrifound dark red (ADR), Agrifound white (WO) and NHRDF-Red (L28) were taken and their quercetin content in extracts and fractions of all three varieties was determined by HPTLC and the method proposed was duly validated in accordance with ICH guidelines of method validation [19, 20]

EXPERIMENTAL

Materials and chemicals. Three onion varieties i.e. ADR, WO, L28 were procured from National Horticulture Research and Development Foundation, India during the month of November, 2012. These varieties were identified and authenticated by Dr. L.R. Verma, Deputy Director NHRDF, Regional Research Station, Karnal (Haryana). Analytical-grade solvents were obtained from E-Merck, Mumbai, India.

Preparation of Methanol Extracts. Methanol extract of shade dried outer scales of onion bulbs of all three varieties was prepared. Dried outer scales (100 gm) were ground with 90% methanol and kept in an ultrasonic bath for 30 min. and then allowed to stand for 24 h at room temperature. After 24 h supernatant was collected and filtered. The solvent was removed under reduced pressure using rotatory vacuum evaporator with bath temperature around 35-40°C¹⁸. The weight of the extract was calculated on the dry weight basis.

Preparation of Flavonoid rich fraction (FRF). Flavonoid rich fraction was prepared from the methanol extract of outer scales of all three varieties of onion bulbs. Dried methanol extract was suspended uniformly in water and placed in three-

necked round bottom flask and fractionated with ethyl acetate for 30 minutes at 50°C with continuous stirring, this procedure was repeated for 5 to 7 times and then all the ethyl acetate fractions were pooled and dried under reduced pressure to give flavonoid rich fraction (FRF)²¹.

Instrumentation and chromatographic conditions.

Camag (Muttens, Switzerland), HPTLC system including a Linomat V sample applicator, Camag twin-trough plate development chamber, Camag TLC scanner 4 and Wincats integration software was used. Precoated Aluminium backed HPTLC plates 20 x 10 cm with 0.2 mm layer of silica gel 60 F₂₅₄ (Merck), prewashed with methanol, were used. Quercetin was used as marker, and a standard solution of 1 mg/ml was prepared to get six dilutions of different concentrations (100 ng, 200 ng, 300 ng, 400 ng, 500 ng, 600 ng). A volume of 10 µl from each dilution was applied in triplicate on pre-coated TLC plate using Linomat V TLC plate applicator at about 1 cm above the edge using bandwidth of 8 mm and distance between two tracks 10 mm. The chromatogram was developed under chamber saturation with the selected solvent system i.e. Toluene: Ethyl acetate: Formic Acid (8.5: 5.5: 1), to a distance of 8 cm. The plate was dried and visualized in UV cabinet 254/366 nm. The profiles, after visualization, were recorded as an image (Fig. 1). Then the plate was scanned with Camag TLC Scanner 4 at a wavelength of 254 nm.

Preparation of calibration curve of standard. A calibration curve of standard quercetin was prepared using quercetin (1 mg/ml) between concentration of quercetin and area under the curves (AUCs) by applying 10 µl of each of different concentrations i.e. 100 ng, 200 ng, 300 ng, 400 ng, 500 ng, 600ng of standard quercetin solution (Fig. 2). This calibration curve was used in calculating the amount of quercetin in extracts/fractions of three different onion varieties.

Method validation studies

The quantitative TLC method was validated for linearity, sensitivity, precision and accuracy for marker. The linearity, range, limit of detection, limit of quantification, inter day precision, intra-day precision, accuracy was observed (Table 2).

Instrument precision

Instrumental precision was checked by repeated scanning (n=7) of the same spot of quercetin (300 ng/spot) expressed as % coefficient of variance (% CV).

Repeatability

The repeatability of the method was affirmed by analyzing 300 ng/spot of quercetin individually on a TLC plate (n=5) and expressed as % CV.

Intra-day and Inter-day variation

Variability of the method was studied by analyzing aliquots of standard solution containing 300 ng/spot of quercetin on same day (Intra-day precision) and on different days (Inter-day precision).

Limit of detection (LOD) and Limit of quantification (LOQ)

LOD and LOQ were determined by applying different concentrations of standard solutions of quercetin along with methanol as blank and determined on the basis of signal-to-noise ratio. LOD was determined at S/N of 3:1 and LOQ at S/N of 10:1.

Recovery studies

The accuracy of the method was assessed by performing recovery studies at three different levels (50, 100 and 150 % addition of quercetin). The % age recovery and average % were calculated (Table 3).

Specificity

This was ascertained by analyzing the standard compound and sample. The band for quercetin from sample solutions was confirmed by comparing the R_f and spectra of the bands to those of the standard. The peak purity of quercetin was analyzed by comparing the spectra at three different levels, i.e., start middle and end positions of the bands.

RESULT AND DISCUSSION

In present study extract of all three varieties showed the presence of quercetin on TLC plate at same R_f value as that of pure quercetin with mobile phase consisting of toluene: ethyl acetate: formic acid in the ratio of 8.5:5.5:1 (Fig. 1).

The selected mobile phase efficiently resolved the quercetin from other components of onion. The R_f value of quercetin was found to be 0.60 as shown in chromatographs (Fig 3). The calibration plot was linear in range of 100-600 ng of quercetin with a correlation coefficient of 0.997 suggesting linear dependence of peak area on concentration. The calibration curve was represented by the linear equation $y = 5.3814x - 296$, where y is the response as peak area and x is the concentration (Fig. 2).

The content of quercetin in various extracts i.e. outer scale methanol extract (OSME) and flavonoid rich fraction (FRF) outer scales of three different onion

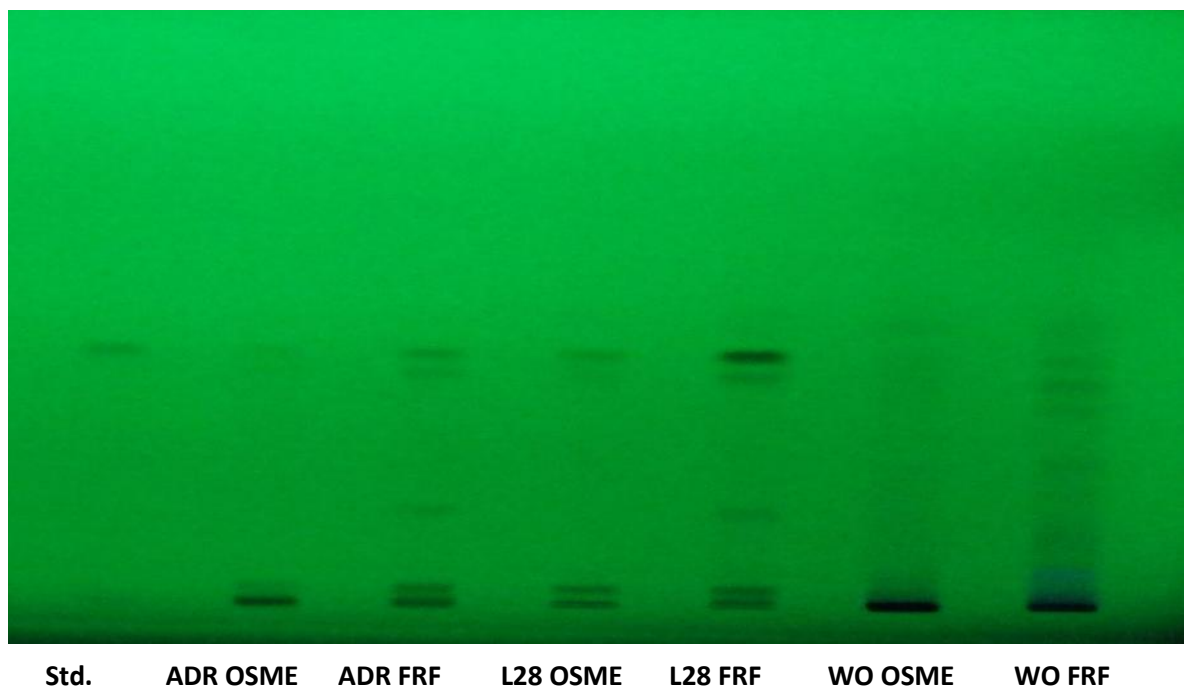
varieties was also calculated using calibration plot and the results are shown in table 1. The maximum amount of quercetin among all varieties was found in methanol extract and flavonoid rich fraction of L28 variety.

TLC densitometric method was validated as per ICH guidelines. The results of parameters for method validation like inter and intraday precession, repeatability, limit of detection (LOD) and limit of quantification (LOQ) and instrumental precession are given in table 2. To ascertain the effectiveness of method recovery studies was performed and the extracts were spiked at 50, 100 and 150 % level with pure quercetin. The results of the recovery studies are shown in table 3. The recovery values obtained was in range of 97.30-98.48 % and the overall average recovery was found out to be 98.20 % indicating the reliability and reproducibility of the method.

The chromatograph showing the R_f values of quercetin and FRF of L28 is presented in fig. 3. The spectral overlay of quercetin and test extracts is shown in fig. 4.

Onion ranked highest in quercetin content in a survey of 28 vegetables and 9 fruits and according to one report it provides around 29% of flavonoids consumed in the Dutch diet^{22,23}. Quercetin has been reported to exhibit anxiolytic activity using *in vitro* test²⁴; antidepressant activity using FST²⁵; antistress activity using FST²⁶; analgesic activity using acetic acid induced pain test²⁷. In some of the recent studies it has been shown that quercetin leads to improvement in memory in aged mice and delayed the deterioration in memory at early stages of Alzheimer's disease (AD)²⁸. Also, in one more very recent study it has been reported that co-administration of vitamin E plus quercetin with Cyclosporine A (CsA) in renal transplant patients may be beneficial in reducing the nephrotoxic effects of CsA²⁹. Thus, quercetin was used as markers to standardize the plant. The content of quercetin was determined using validated TLC densitometric method. The validation parameters compiled with the specified limits.

Quercetin in onions exists predominantly in three forms i.e quercetin aglycone, quercetin-3,40-O-diglucoside, and quercetin-40-O-glucoside³⁰. Since, amounts of quercetin in onions vary with bulb color and type and mostly being distributed in the outer skins and rings^{31,32}, we choose to prepare extracts and fraction of outer scale of three different varieties of onion to standardize a method for comparative evaluation using High Performance Thin Layer Chromatography (HPTLC).



(ADR - Agrifound dark red; WO-Agrifound white; L28-NHRDF-Red varieties of onion
OSME-Outer scales methanol extract; FRF-flavonoid rich fraction)

Fig. 1

Comparative TLC fingerprint profile of methanol extracts and flavonoid rich fractions.

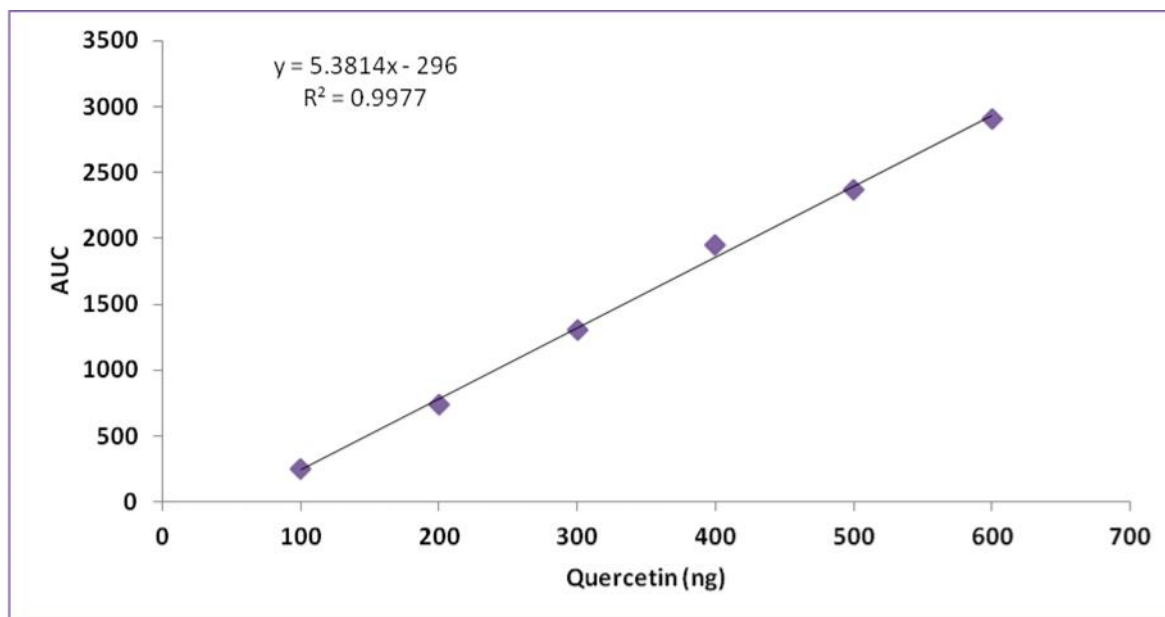
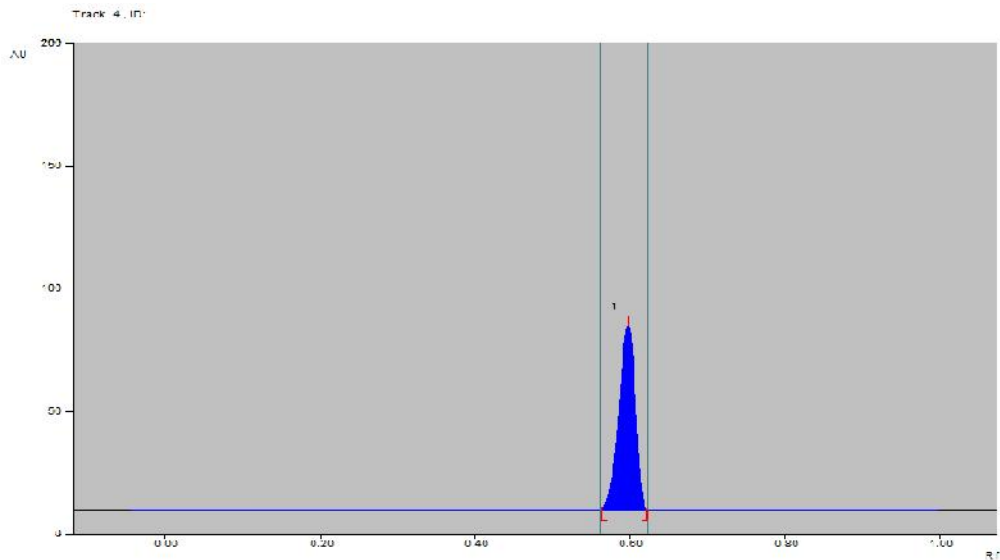
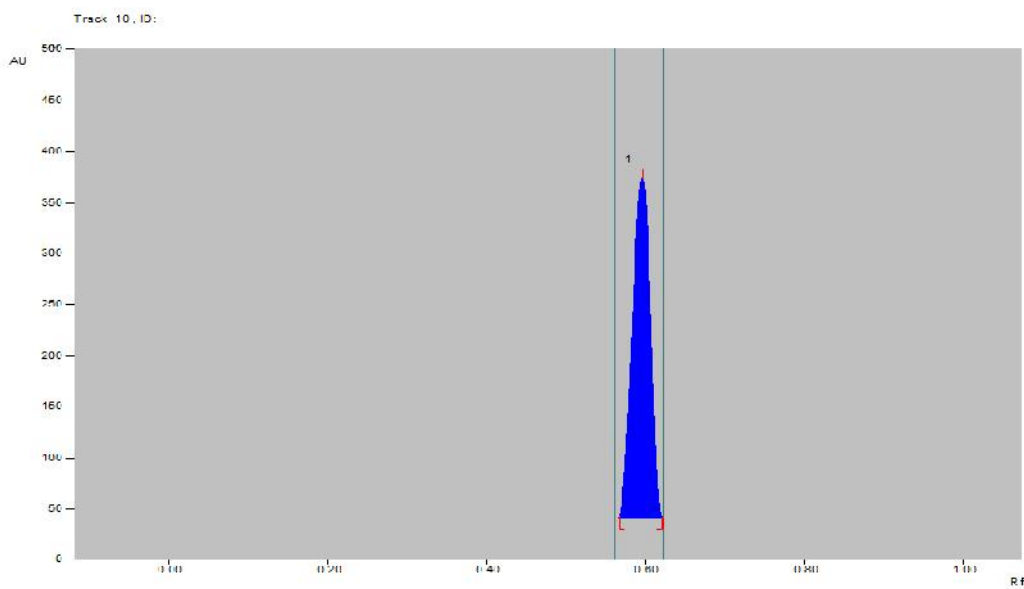


Fig. 2

Standard plot of quercetin in TLC densitometric analysis.



(A)



(B)

Fig. 3
Chromatograph showing the Rf values of (A) Standard Quercetin; (B) FRF of L28 variety.

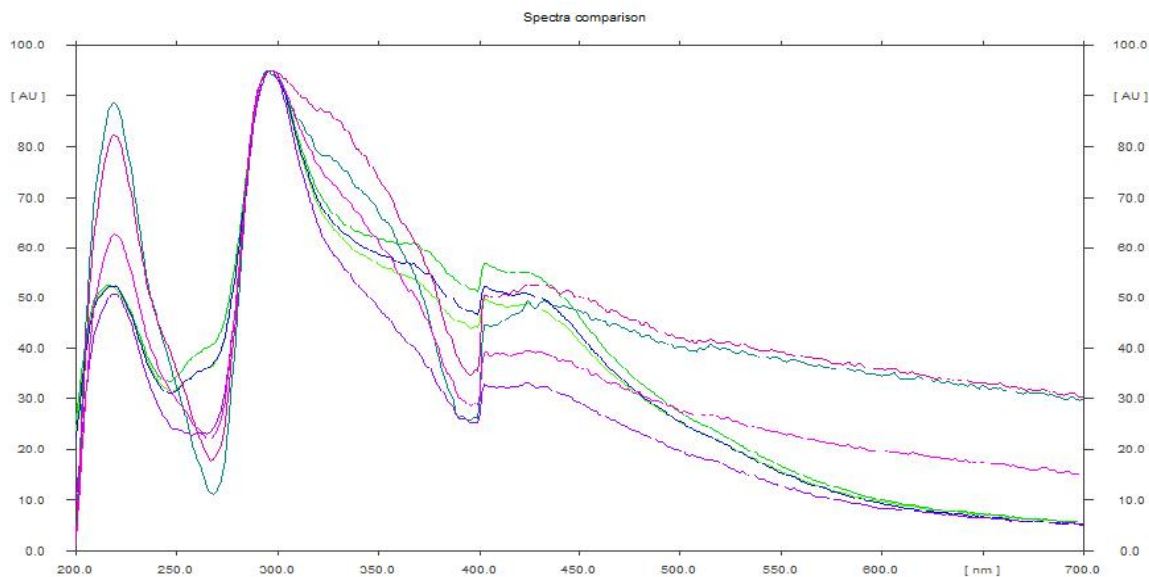


Fig. 4
Spectra overlay of quercetin with corresponding peak in methanol extracts and flavonoid rich fractions of *A. cepa* varieties.

Table 1
Percentage yield of quercetin in *A. cepa* outer scales.

Plant variety	Extract	Percentage yield of Quercetin in plant (% w/w) \pm S.D.	Percentage yield of Quercetin in extracts (% w/w) \pm S.D.
ADR	OSME	0.0252 \pm 0.0062	1.52 \pm 0.020
	FRF	0.0633 \pm 0.0025	8.25 \pm 0.040
L-28	OSME	0.1676 \pm 0.0045	9.21 \pm 0.030
	FRF	0.2065 \pm 0.0052	25.30 \pm 0.080
WO	OSME	0.0006 \pm 0.0012	0.01 \pm 0.001
	FRF	0.0100 \pm 0.0034	0.33 \pm 0.04

Table 2
Method validation parameters in TLC-densitometric analysis of *A. cepa*.

S. No.	Parameter	Values
1	Instrumental precision (% CV, n=7)	0.75
2	Repeatability (% CV, n=5)	0.99
3	Linearity (coefficient of correlation)	0.997
4	Range (ng)	100-600
5	LOD (ng)	10
6	LOQ (ng)	35
7	Intra-day precision (% CV, n=9)	1.4
8	Inter-day precision (% CV, n=9)	1.8
9	Accuracy (average % recovery)	98.20
10	Specificity	Specific

Table 3
Recovery studies of Quercetin.

Compound	Initial amount		Amount detected (mg)	Per cent recovery	Average percent recovery
	In percent	In recovery			
Quercetin	0.2065	0.1032 (50%)	0.3050	98.48	98.20
		0.2065 (100%)	0.4060	97.30	
		0.3097 (150%)	0.5050	97.83	

HPTLC is a sophisticated instrumental technique with merits of easy method development and validation, automation, scanning, full optimization, selective detection principle, minimum sample preparation, etc., enable it to be a powerful analytical tool for quantitative determination of particular compound(s) in complex mixtures of inorganic, organic and biomolecules³³.

CONCLUSION

Onions are a rich source of flavonoids; the most prominent of which is quercetin. In this study a simple and precise HPTLC method for the quantitative determination of this marker was developed and validated. The developed method demonstrated good reliability and reproducibility. This may be used in future to evaluate the quality of different varieties of onions with respect to the quercetin content.

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