# Estimation of *trans*-Resveratrol in Herbal Extracts and Dosage Forms by High-Performance Thin-Layer Chromatography[1)]

Sukala Kanna Babu, Katta Vijaya Kumar, and Gottumukkala Venkata Subbaraju*

*Laila Impex Research Centre, Unit I, Phase III; Jawahar Autonagar, Vijayawada–520 007, India.*
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**A simple, sensitive and precise high-performance thin-layer chromatographic (HPTLC) method of analysis of *trans*-resveratrol in *Polygonum cuspidatum* root extracts and in dosage forms was developed and validated. The separation was carried out on a TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase, eluted with chloroform–ethylacetate–formic acid (2.5 : 1 : 0.1) as mobile phase. Densitometric analysis of *trans*-resveratrol was carried out in the absorbance mode at 313 nm. This system was found to give compact spot for *trans*-resveratrol (*Rf* value of 0.40±0.03). A good linear regression relationship between peak areas and the concentrations was obtained over the range of 0.5—3.0 μg/spot with correlation coefficient 0.9989. The limit of detection and quantification was found to be 9 and 27 ng/spot. The method was validated for precision and recovery. The spike recoveries were within 99.85 to 100.70%. The RSD values of the precision in the range 0.37—1.84%. The proposed developed HPTLC method can be applied for identification and quantitative determination of *trans*-resveratrol in herbal extracts and dosage forms.**

**Key words** high-performance thin-layer chromatography; *trans*-resveratrol; dosage form

*trans*-Resveratrol [(*E*)-5-[2-4(hydroxyphenyl)ethenyl]-1,3-benzenediol] is a naturally occurring polyphenolic compound belonging to a group called stilbenes, found in grapes, peanuts and other plants.[2)] *trans*-Resveratrol is a strong antioxidant and reported to have a protective effects against atherosclerosis, coronary heart disease, postmenopausal problems, inhibits platelet aggregation and a broad spectrum of degenerative diseases[3)] and also possess cancer chemopreventive properties.[4)] The roots of *Polygonum cuspidatum*, (Polygonaceae), an important Traditional Chinese Medicinal plant, are widely used for the treatment of various ailments in East Asian countries, and a rich source of *trans*-resveratrol.[5)]

Because of the significant pharmacological activities exhibited by the resveratrol, several researchers have focused on the development of various analytical methods to determine resveratrol in different matrices such as plant extracts, wine and serum. These methods include gas chromatography-mass spectrometry (GC-MS),[6)] liquid chromatography-mass spectrometry (LC-MS),[7)] high-performance liquid chromatography (HPLC) based on UV absorption, fluorimetric and electrochemical detection[8—12)] and the capillary electrophoresis (CE)[13,14)] techniques.

Recently, high-performance thin-layer chromatography (HPTLC) has become a routine analytical technique due to its advantages of its reliability and cost effectiveness.[15,16)] The major advantage of HPTLC is that several samples can be analyzed simultaneously using a small quantity of mobile phase. In the present study, an accurate, specific and reproducible HPTLC method has been developed and validated[17)] for determination of *trans*-resveratrol in *Polygonum cuspidatum* root extracts and dosage forms.

## Experimental

**Materials** Standard *trans*-resveratrol (99%) was procured from Sigma, U.S.A. *Polygonum cuspidatum* root extracts were provided by M/s Laila Impex, Vijayawada, India. *trans*-Resveratrol capsules and tablets were procured from U.S.A. All chemicals and reagents used were of analytical grade and were purchased from Qualigens, India.

**HPTLC Instrumentation** The samples were spotted in the form of bands with a Camag microlitre syringe on a precoated silica gel aluminium plates 60F-254 (20 cm×10 cm with 250 μm thickness, E. Merck, Darmstadt, Germany) using a Camag Linomat IV (Muttenz, Switzerland) applicator. The plates were prewashed by methanol and activated at 60 °C for 5 min prior to chromatography. Linear ascending development was carried out in 20 cm×10 cm twin trough glass chamber (Camag Muttenz, Switzerland) using mobile phase consists of chloroform–ethylacetate–formic acid (2.5 : 1 : 0.1). The length of chromatogram run was 8 cm. Subsequent to the scanning, TLC plates were air dried and scanning was performed on a Camag TLC scanner III in absorbance mode at 313 nm and operated by Cats software 4.03 version. Evaluation was via peak areas with linear regression.

**Calibration Curve of Standard *trans*-Resveratrol** One milligram per milliliter *trans*-resveratrol standard stock solution was prepared in methanol. Standard working solutions were prepared by diluting stock solution with methanol in the concentration range 100—600 μg/ml. Five microliters from each standard solution was spotted on the TLC plate to obtain final concentration range of 0.5—3.0 μg/spot. Each concentration was spotted six times on the TLC plate.

**Estimation of *trans*-Resveratrol in Herbal Extracts** To determine the content of *trans*-resveratrol in herbal extracts, 50 mg was transferred in to a 100 ml volumetric flask containing 50 ml methanol, sonicated for 10 min and diluted to 100 ml with methanol. Filtered on a whatman no. 1 filter paper, an aliquot of sample 5 μl was applied on the TLC plate. The analysis was repeated for six times.

**Estimation of *trans*-Resveratrol in Marketed Dosage Forms** To determine the content of *trans*-resveratrol in capsules and tablets, the average weights were determined by weighing 20 capsules/tablets and they were finely powdered. The weight of the powder equivalent to labeled amount of *trans*-resveratrol transferred in to a 100 ml volumetric flask containing 50 ml methanol, sonicated for 10 min and diluted to 100 ml with methanol. Filtered on a whatman no. 1 filter paper, an aliquot of sample 5 μl was applied on the TLC plate. The analysis was repeated for six times.

## Results and Discussion

The composition of the mobile phase for TLC was optimized by testing different solvent mixtures of varying polarity. The best results were obtained using chloroform–ethylacetate–formic acid (2.5 : 1 : 0.1). The selected mobile phase showed good resolution (Fig. 1). The compound with *Rf* value 0.40±0.03 was identified as *trans*-resveratrol.

**Method Validation** The method for quantitative analysis of *trans*-resveratrol was validated with regard to its specificity, precision, accuracy and linearity.

* To whom correspondence should be addressed. e-mail: subbarajugottumukkala@hotmail.com

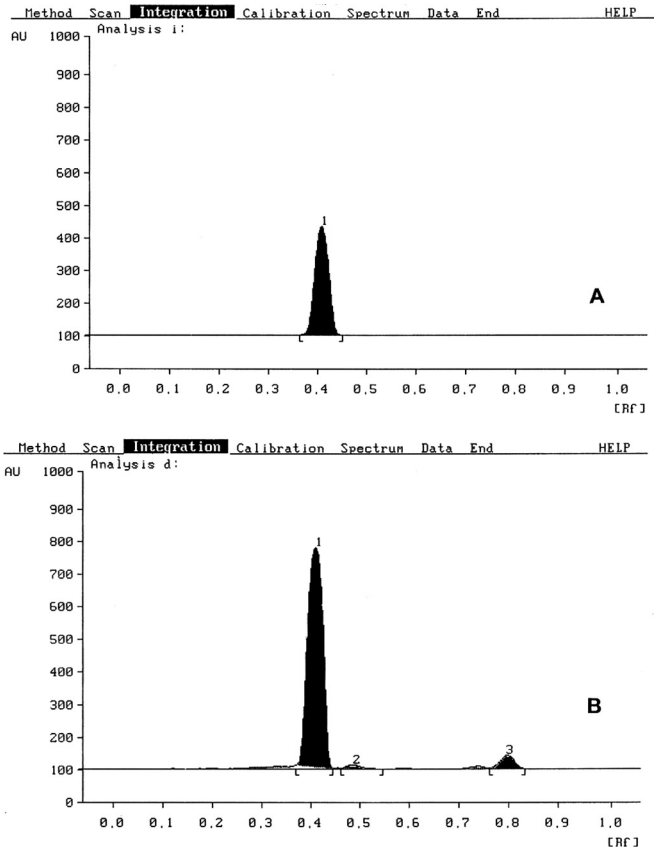


Fig. 1. HPTLC Chromatograms of (A) Standard *trans*-Resveratrol (B) a Typical Root Extract of *Polygonum cuspidatum*

Key to peak identity: 1, *trans*-resveratrol.

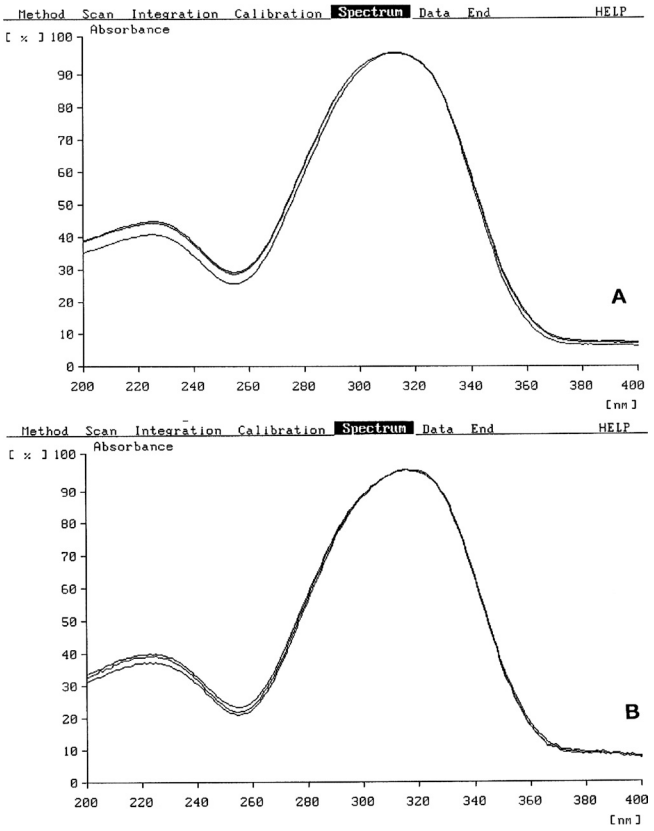


Fig. 2. Overlay Spectra of (A) Standard *trans*-Resveratrol (B) Sample at Peak Start, Peak Maximum and Peak End in Absorbance Mode in the UV Range, Taken on the CAMAG TLC Scanner III

Table 1. Intra- and Inter-Day Precision of HPTLC Method ($n=6$)

| S. No. | Sample | Intra-day precision | | Inter-day precision | |
|---|---|---|---|---|---|
| | | % *trans*-resveratrol | RSD% | % *trans*-resveratrol | RSD% |
| 1 | K-31 | 56.50 | 1.54 | 55.90 | 1.07 |
| 2 | K-32 | 53.87 | 1.69 | 52.83 | 1.59 |
| 3 | K-33 | 53.30 | 1.84 | 53.38 | 0.37 |

Table 2. Recovery Studies ($n=6$)

| S. No. | Amount of *trans*-resveratrol added (mg) | Amounts of *trans*-resveratrol recovered (mg)±S.D. | % Recovery |
|---|---|---|---|
| 1 | 1.0000 | 1.0070±0.05 | 100.70 |
| 2 | 2.0000 | 1.9970±0.06 | 99.85 |
| 3 | 3.0000 | 3.0040±0.12 | 100.13 |

The specificity of the method was ascertained by analyzing standard and samples. The spot for *trans*-resveratrol in the sample was confirmed by comparing the *Rf* value and the spectrum of the spot with that of standard. Peak purity of the sample was fully in confirmity with the standard which showed in Fig. 2.

The reproducibility of the method was determined by different analysts using the samples from the same homogeneous batch and repeatability was determined by intra-day and inter-day precision, expressed in terms of percent relative standard deviation (RSD%) or coefficient of variation (CV). Six determinations were carried out on the same sample, on the same day for intra-day and over two consecutive days for inter-day precision on three samples. The RSD% values of intra-day and inter-day were between 1.54—1.84% and 0.37—1.59%, respectively (Table 1). No significant intra- and inter-day variation was observed in the analysis of *trans*-resveratrol in three different samples. The RSD% of the reproducibility of the method was found to be <2%.

The accuracy of the method was determined from recovery studies. A known but varying amounts of standards from *trans*-resveratrol was added to the pre-analyzed sample and analyzed according to the procedure. The results were reported in Table 2. The average recovery percentage value was found to be 100.23±0.43.

Linearity was evaluated by determining six standard working solutions containing 100—600 μg/ml of *trans*-resveratrol. Peak area and concentrations were subjected to least square linear regression analysis to calculate the calibration equation and correlation co-efficient. Linearity was found over concentration range 0.5—3.0 μg/spot with a correlation co-efficient ($r$) 0.9989. The linearity of calibration graph and adherence of the system to Beer's law was validated by high value correlation co-efficient.

In order to estimate the limit of detection (LOD) and limit of quantification (LOQ), blank methanol was spotted six times. LOD and LOQ were determined based on the standard deviation of the response of blank and slope estimated from

Table 3. Estimation of *trans*-Resveratrol by Proposed HPTLC Method ($n=6$)

| S. No. | Sample | Labeled amount (mg) | Estimated *trans*-resveratrol±S.D. |
|---|---|---|---|
| 1 | Herbal extracts[a)] | | |
| | K-31 | — | 56.50±0.87[c)] |
| | K-32 | — | 53.87±0.91[c)] |
| | K-33 | — | 53.30±0.98[c)] |
| 2 | Dosage forms[b)] | | |
| | Tablet 1 | 13.00 | 12.21±0.20 |
| | Tablet 2 | 20.00 | 18.93±0.18 |
| | Tablet 3 | — | 4.56±0.09 |
| | Capsule 1 | 10.00 | 9.70±0.14 |
| | Capsule 2 | 15.00 | 14.14±0.25 |

*a*) K-31, K-32, K-33: commercial *Polygonum cuspidatum* root extracts supplied by M/s Laila Impex, Vijayawada, India. *b*) Tablet 1, KAL–Resveratrol; Tablet 2, Source Naturals–Resveratrol; Tablet 3, Jarrow Formulas–Resveratrol Synergy® (contains 16 mg of total resveratrol per tablet); Capsule 1, Natrol–Protykin®–Resveratrol; Capsule 2, Solaray–Resveratrol. *c*) Expressed as mg/100 mg of extract.

the calibration curve of the *trans*-resveratrol. The LOD and LOQ was found to be 9 ng/spot and 27 ng/spot for *trans*-resveratrol.

**Application of the Method** The method was applied for the estimation of *trans*-resveratrol in three different *Polygonum cuspidatum* root extracts, three different tablets and two different capsules, available in the market. One spot of *Rf* 0.40±0.03 was observed in chromatogram of the analyte isolated from the extracts, tablets and capsules along with other components. There was no interference in the analysis of *trans*-resveratrol from other components. These components appear in the chromatogram at significantly different *Rf* values. The results obtained by the proposed HPTLC method was presented in Table 3.

Interference studies revealed the presence of other polyphenolic compounds usually present in the herbal extracts, ascorbic acid and general excipients used in dosage forms does not interfere in the estimation of *trans*-resveratrol.

## Conclusions

The developed HPTLC method is simple, specific and accurate. Statistical analysis proves that the method is reproducible and selective. This method can be used for the quantitative determination of *trans*-resveratrol in herbal extracts and dosage forms.

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