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Development and Validation of HPTLC Method for Simultaneous Estimation of Resveratrol and Piperine in Its Pharmaceutical Dosage Form

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Resveratrol is a plant compound that work as antioxidant and it also has anti-aging properties whereas Piperine is a alkaloid and it majorly used in spices. A HPTLC (High performance thin layer chromatography) study is conducted for estimation of Resveratrol and Piperine. The Mobile phase used was Chloroform: Ethyl Acetate (50:50 v/v). Rf Value of Resveratrol 0.59 and Piperine 0.79 was found. Stationary phase of Silica gel 60 F_{254} was used. Densitometric analysis was performed at 325 nm. The method was found to be linear. Recovery (ranging from 97% to 102.24 %), limit of detection (40.26 ng/spot, 1.64 ng/spot respectively for resveratrol & piperine), limit of quantification (122.02 ng/spot, 4.98 ng/spot respectively for resveratrol & piperine) and precision (≤ 2.00%) were found to be satisfactory. Validation performed with linearity, accuracy, precision, specificity, robustness, limit of detection and limit of quantification. Every parameter found within the range. The developed method allows to confirm that an accurate and reliable potency measurement of a pharmaceutical preparation can be performed.

Keywords: HPTLC; piperine; resveratrol; validation.

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1. INTRODUCTION

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) belongs to polyphenols' stilbenoids group, possessing two phenol rings linked to each other by an ethylene bridge. This natural polyphenol has been detected in more than 70 plant species, especially in grapes' skin and seeds, and was found in discrete amounts in red wines and various human foods. It is a phytoalexin that acts against pathogens, including bacteria and fungi. As a natural food ingredient, numerous studies have demonstrated that resveratrol possesses a very high antioxidant potential. Resveratrol also exhibits antitumor activity, and is considered a potential candidate for prevention and treatment of several types of cancer. Indeed, resveratrol anticancer properties have been confirmed by many in vitro and in vivo studies, which shows that resveratrol is able to inhibit all carcinogenesis stages (e.g., initiation, promotion and progression). Even more, other bioactive effects, namely as anti-inflammatory, anticarcinogenic, cardioprotective, vasorelaxant, Phyto estrogenic and neuroprotective have also been reported [1].

Piperine is a constituent of black and white pepper belongs to the family Piperaceae. Piperine is reported for diverse therapeutic actions like central nervous system depressant, analgesic [2] inhibition of hepatic drug metabolism [3] enhancing pentobarbitone induced hypnosis [4] bioavailability of oxyphenbutazone [5] hepatoprotective activity [6] anti-inflammatory activity [7] inhibition of lipid peroxidation during experimental inflammation [8] devoid of genotoxic effects [9] antifertility [10] and antidiarrheal [11] It also possesses radioprotective effects [12].

In the market combined dosage form of Resveratrol and Piperine is available. Hence to standardized this formulation, in present study HPTLC method for estimation of Resveratrol and Piperine was developed.

In the present study attempt has been made to developed simple, precise and accurate HPTLC method for simultaneous estimation of Resveratrol and Piperine to standardized herbal formulation.

2. MATERIALS AND METHODS

2.1 Instrumentation

A CAMAG HPTLC system (CHF7150) comprising of Linnomat-5 (sample applicator), CAMAG TLC scanner, CAMAG WINCAT software and Hamilton Syringe (100 µL) were used during analysis. Precoated TLC silica gel 60 F_{254} were used for analysis.

2.2 Chemicals and Reagents

Standard Resveratrol and Piperine were procured from Sigma Aldrich, Mumbai. Other chemical used during analysis were purchased from Merck and of AR grade.

2.3 Chromatographic Condition

Stationary Phase: Pre coated Silica gel plate 60 F_{254} pre washed with Methanol
Mobile Phase: Chloroform: Ethyl acetate (50:50 v/v)
Distance Between Bands: 14 mm
Separation Technique: Ascending development
Scanning mode: Absorbance
Detection Wavelength: 326 nm
Scanning Speed: 10 nm/sec
Band Length: 6mm

2.4 Preparation of Sample Solution

For analysis of sample, twenty capsules were taken and individually weighed then their average weight (19.53 gm) was calculated. The granules were separated from capsule shell and triturated to get uniform powder, powder equivalent to 10 mg was weighed and transferred to a 10 mL volumetric flask, to it 5 mL of methanol was added and sonicated for 10 min. Volume was made up to 10 ml with methanol to make the concentration of 10mg/mL. This solution was filtered and used for further analysis.

2.5 Standard Resveratrol and Piperine Solution

Standard solution of Piperine and Resveratrol was prepared by dissolving the 10 mg and 25 mg of drug into 10 ml of methanol respectively, which gives solution of concentration 1 mg/mL and 2.5 mg/mL, necessary dilutions were made for further analysis.

2.6 Selection of Wavelength

For the UV spectra solutions of R and P in the concentration of 10 ppm were scan over a range 200-400 nm in UV spectrophotometer. After scanning spectra obtained were overlaid and maximum absorption wavelength was selected for analysis [13] (Fig. 1).

2.7 Optimization of Mobile Phase

To get well resolved peaks different solvents likes Chloroform, Ethyl acetate, Formic acid, Toluene, Glacial acetic acid, Diethyl ether etc. were used in single and in different composition. After taking composition the Mobile phase was finalized which gave the resolved peaks.

2.8 Calibration

TLC plates of uniform thickness were pre-washed by methanol and then activation was done in hot air oven. Aliquot of Standard solution of Resveratrol and Piperine was applied in 0.2 µL, 0.4 µL, 0.6 µL, 0.8 µL and 1.2 µL respectively, over the silica gel 60F 254 plate. The plates were dried in air and scanned by using CAMAG TLC Scanner. A calibration equation relating to the standard concentration to scan areas was determined [14].

2.9 Method Validation

After the method development, the method validation was performed with some parameter as per ICH guideline Q2 R1. Method validation gives assurity of analysis which was suitable for its use. It includes Linearity, Accuracy, Precision, Robustness, Specificity, LOD and LOQ.

2.10 Accuracy and Precision

Accuracy can be observed by closeness between accepted reference value and the value found. Accuracy of method were confirmed by performing recovery studies. Precision was performed by taking 3 determinations of Interday and Intraday, which gives results of precise method and % RSD were also calculated.

2.11 Linearity

The range of results which was proportional to Concentration of analyte in sample showed the relation with absorbance. Linearity was carried out by using concentration50-300 ng/band and 2-12 ng/band for both Resveratrol and Piperine respectively. The r^2 were calculated by plotting graph of concentration vs absorbance.

2.12 Robustness

Robustness was determined bychanges in the mobile phase like in its composition, volume and duration of chamber pre-saturation. It was done in six replicates of concentration in ng/band.

2.13 Specificity

In specificity, comparison was done betweenspectra of Methanol, Mobile phase, Standard and Sample solution.

2.14 LOD and LOQ

Limit of Detection and limit of quantification of Resveratrol and Piperine was calculated manually. By using following formula –

$$Limit\ of\ Detection\ (LOD) = \frac{3.3 * \sigma}{\text{Slope}}$$

$$Limit\ of\ Quantification\ (LOD) = \frac{10 * \sigma}{\text{Slope}}$$

3. RESULTS AND DISCUSSION

Optimization of Mobile phase, after screening of different mobile phase, solvent system of Chloroform: Ethyl acetate (50:50 v/v) was used for separation of Resveratrol and Piperine. This mobile phase gave well resolved peaks of Resveratrol and Piperine shown in Fig. 2. The linearity of developed method was performed by taking different concentrations of Resveratrol and Piperine. From the graph, the value of r^2 was observed to be 0.9978 for Resveratrol and the value of r^2 was observed to be 0.9960 for Piperine, given in the Table 1 and Fig. 3. The drug content as per label claim was found to be 99.1 % for Resveratrol and 98.7 % for Piperine is given in the Table 2.The recovery of Resveratrol and Piperine was explained in terms of SD and % RSD. The percentage recovery values were observed in following range, from 94.97 to 100.32 for Resveratrol and 99.05 to 102.43 for Piperine. The result of recovery study are given in Table 3. For Resveratrol and Piperine, Relative standard deviation of all the parameters is less than 2%. To measure precision of Intraday and Interday, six samples of concentration were taken and % of Relative Standard Deviation calculated and it reveals that, the method is precise, which is given in Table 4. Robustness done by keeping all parameter constant except mobile phase volume, saturation time and its composition, and calculated % of Relative Standard Deviation were within the acceptance limit given in Table 5. Specificity was carried out by taking some different peak (Peak of methanol and mobile phase)along with peak of standard and sample solution. The standard and sample peak spectra were showing the

specificity Fig. 4, it shows trials of methanol, mobile phase, standard and sample solution. Limit of Detection for Resveratrol and Piperine were found 40.26ng and 1.64ng and Limit of Quantification were 122.02ng and 4.98ng respectively.

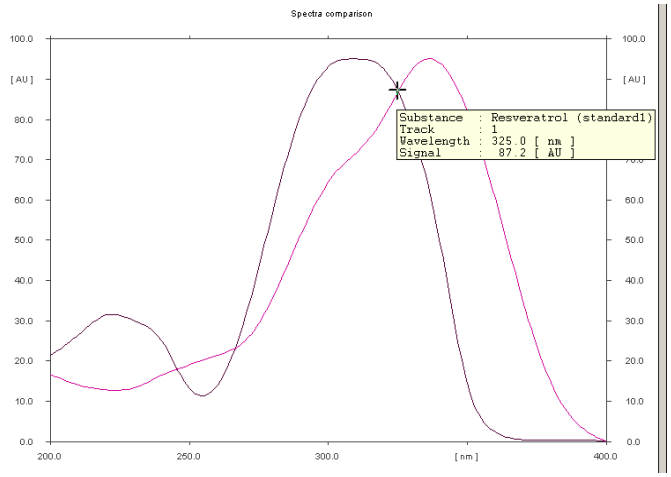

Fig. 1. Selection of Wavelength

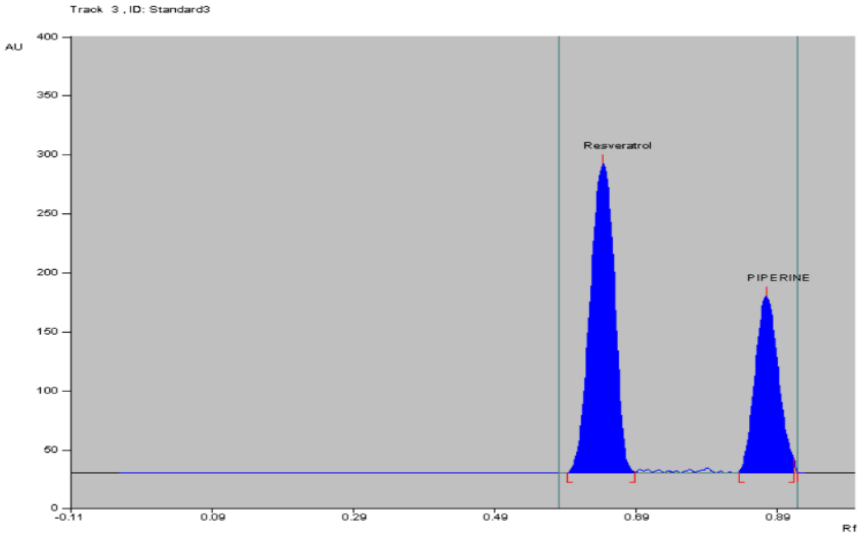

Fig. 2. Densitogram of resveratrol and piperine

Table 1. Regression parameters for the analysis of resveratrol and piperine

Parameter	Resveratrol	Piperine
Rf	0.59	0.79
Regression Equation	y = 45.771x + 661.81	y = 495.59x + 1430.6
Regression Coefficient	0.9978	0.9960
Slope	661.81	1430.6
Intercept	45.771	495.59

Table 2. Drug content of resveratrol and piperine in marketed formulation

Drug	Amount Present (mg/Capsule)	Mean Area of Formulation	SD	%RSD	Amount Found	% of drug Found
Resveratrol	250	8447.03	4.1789	0.0494	248.57	99.1
Piperine	1	4403.83	1.4843	0.0337	0.91	98.7

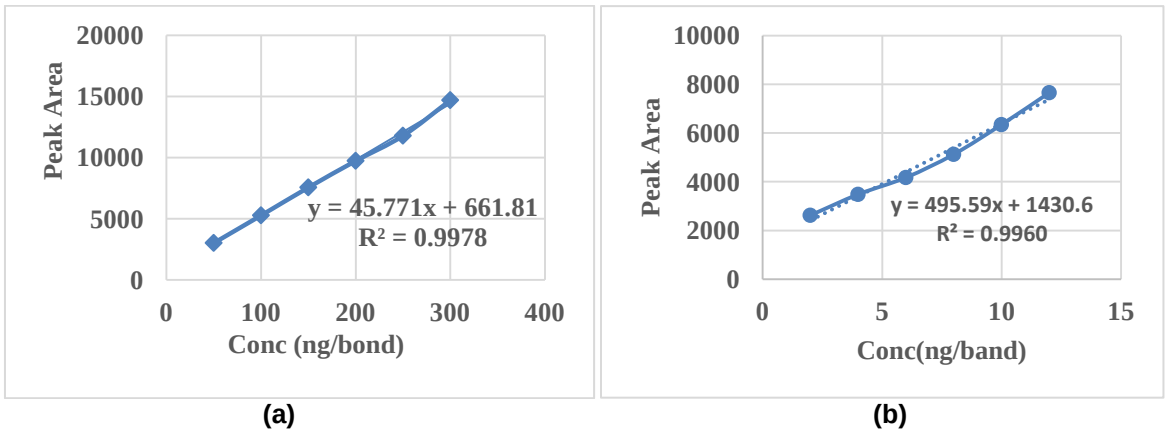

Fig. 3. Calibration Graph for Resveratrol (A) and Piperine (B)

Table 3. Accuracy studies for resveratrol and piperine

Drug	Accuracy Level (%)	Amount Recovered (mg/ml)	% Recovery	SD	%RSD
Resveratrol	80	23.78	94.97	0.0666	0.0701
	100	30.06	97.00	0.2000	0.2062
	120	49.59	100.32	0.1222	0.1218
Piperine	80	0.79	99.05	0.0808	0.0816
	100	1.02	102.24	0.2254	0.2204
	120	1.23	102.43	0.3951	0.3857

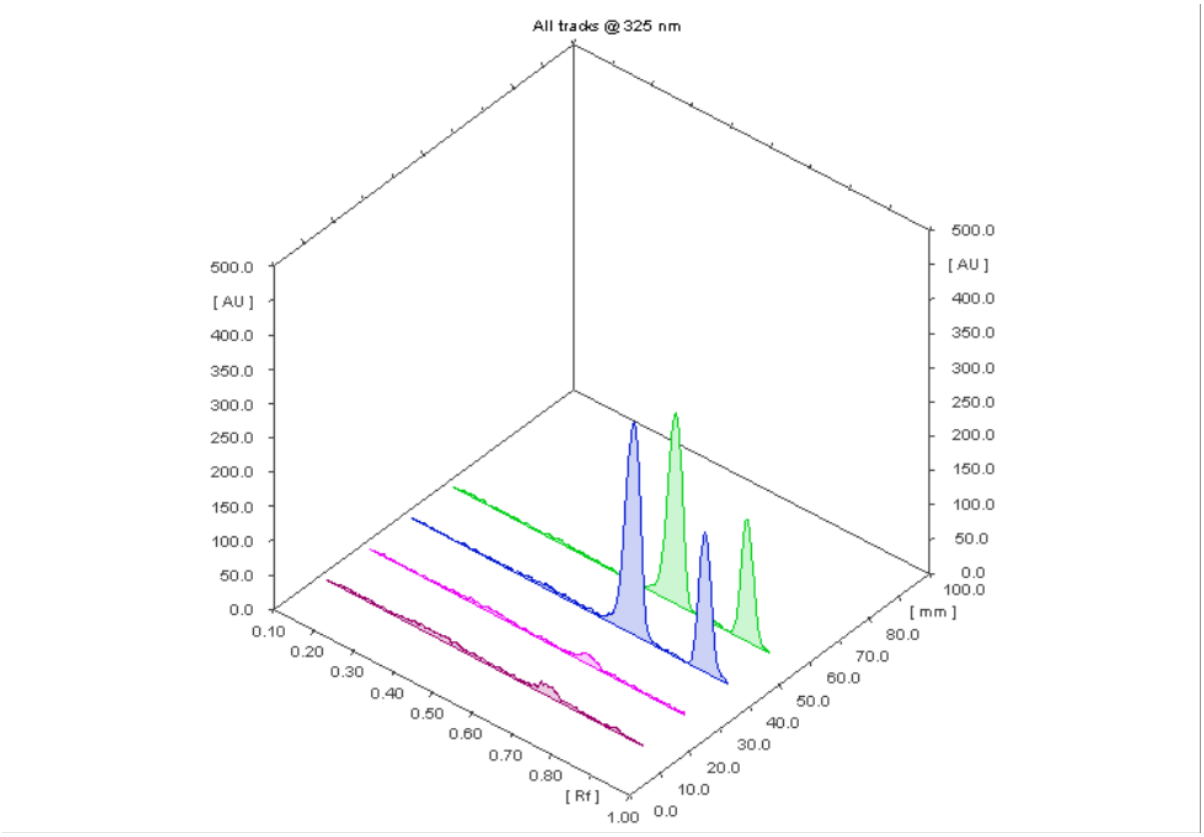

Fig. 4. Specificity of methanol, mobile phase, standard and sample solution

Table 4. Precision of the interday and intraday measurement for resveratrol and piperine

Drug	Concentration (ng/band)	Interday RSD for Peak area (%)	Intraday RSD for Peak area (%)
Resveratrol	50	0.0253	0.0375
	100	0.1304	1.2189
	150	0.6840	0.1042
Piperine	50	1.0874	0.1609
	100	0.5814	0.7457
	150	1.0421	0.2594

Table 5. Robustness (N=6), Concentration 200NG/BAND)

Parameter	Resveratrol (%RSD)	Piperine (%RSD)
Saturation time	1.2628	0.9951
Mobile Phase composition	0.1217	0.22045
Volume of Mobile phase	0.7491	0.8054

Table 6. LOD And LOQ for resveratrol and piperine

Drug	LOD (ng/band)	LOQ (ng/band)
Resveratrol	40.26	122.02
Piperine	1.64	4.98

4. CONCLUSION

A simple, accurate and specific HPTLC method was developed. This method is versatile in the combination analysis of resveratrol and piperine as well as its formulation and in first attempt both markers simultaneously estimated and compared successfully. The HPTLC method shown to be specific, linear, repeatable and accurate, within the established ranges. Hence it can be concluded that this method can be adopted for analysis of resveratrol and piperine.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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