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

Ankita Gaikwad¹, Madhuri Shelar²*, Ganesh Andhale¹ and Jyoti Kadam¹

¹Department of Pharmaceutical Quality Assurance Technology, Alard College of Pharmacy, Pune-411057, Maharashtra, India.

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

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(1) Dr. Giuseppe Murdaca, University of Genoa, Italy.
(2) Emmanuel Obeagu, Imo State University, Nigeria.
(3) Biswajit Dash, NEPEDS College of Pharmaceutical Sciences, India.
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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 F254 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.

*Corresponding author: E-mail: madhurishelar70@yahoo.com;
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 F254 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 F254 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 like 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 concentration 50-300 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 by changes 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 between spectra 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 –

\[\text{Limit of Detection (LOD)} = \frac{3.3 \times \sigma}{\text{slope}}\]

\[\text{Limit of Quantification (LOD)} = \frac{10 \times \sigma}{\text{slope}}\]

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.

4. COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Salehi B, Mishra AP, Nigam M, Sener B, Kilic M, Sharifi-Rad M, Fokou PV, Martins N, Sharifi-Rad J. Resveratrol: A double-edged sword in health benefits. Biomedicines. 2018;6(3):91.
2. Singh N, Kulshrestha VK, Srivastava RK, Kohli RP. A comparative evaluation of piperine and nalorphine against morphine induced respiratory depression and analgesia. J Res Indian Med 1973;8:21-26.
3. Bhat BG, Chandrasekhara N. Effect of black pepper and piperine on bile secretion and composition in rats. Nahrung 1987; 3:913-916.
4. Mujumdar AM, Dhuley JN, Deshmukh VK, Naik SR. Effect of piperine bioavailability of oxyphenylbutazone in rats. Indian Drugs 1999;36:123-126.
5. Mujumdar AM, Dhuley JN, Deshmukh VK, Raman PH, Thorat SL, Naik SR. Effect of piperine on pentobarbitone induced hypnosis in rats. Indian J Exp Biol 1990a; 28:486-487.
6. Mujumdar AM, Dhuley JN, Deshmukh VK, Raman PH, Thorat SL, Naik SR. Effect of
piperine on pentobarbitore induced hypnosis in rats. Indian J Exp Biol. 1990a; 28:486-487.

7. Mujumdar AM, Dhuley JN, Deshmukh VK, Raman PH, Naik SR. Antiinflammatory activity of piperine. Jpn J Med Sci Biol. 1990b;43:95-100.

8. Dhuley JN, Raman PH, Mujumdar AM, Naik SR Inhibition of lipid peroxidation by piperine during experimental inflammation in rats. Indian J Exp Biol. 1993;31:443-445.

9. Karekar VR, Mujumdar AM, Joshi SS, Dhuley JN, Shinde SL, Ghaskadbi SM Assessment of genotoxic effect of piperine using Salmonella typhimurium and somatic and somatic and germ cells of Swiss albino mice. Arzneim-Forsch/Drug Resist Update 1996; 46:972-975.

10. Daware MB, Mujumdar AM, Ghaskadbi S. Reproductive toxicity of piperine in Swiss albino mice. Planta Med 2000;66:231-236.

11. Bajad S, Bedi KL, Singla AK, Johri RK Piperine inhibits gastric emptying and gastrointestinal transit in rats and mice. Planta Med 2001;67:284-287.

12. Aggarwal M, Kaul BL. The radioprotective effect of piperine in plants. Indian Drugs 1992;29:447-449.

13. Marker Based Standardisation of Sarasvata Ghrita, An Ayurvedic Polyherbal Formulation using Rapid Validated High Performance Thin Layer Chromatography Method. Journal of Chemical and Pharmaceutical Research. 2017;9(10): 200-207.

14. Patel RK, Kanani RJ, Patel VR, Patel MG. Development and validation of HPTLC method for simultaneous quantification of vasicine and piperine in Vasavaleha. Int J Pharm Res. 2010;2(3):14-7.