**Pharm Methods, 2015; 6(3): 143-147**
A multifaceted peer reviewed journal in the field of Pharm Analysis and Pharmaceutics
www.phmethods.net | www.journalonweb.com/phm

*Original Article*

# New Derivative and Differential Spectrophotometric Methods for the Determination of Pterostilbene-An Antioxidant

**Mathrusri Annapurna Mukthinuthalapati* and Josyula Sai Phani Kumar**

Department of Pharmaceutical Analysis & Quality Assurance, GITAM Institute of Pharmacy, GITAM University, Visakhapatnam, INDIA-530045.

## ABSTRACT

Pterostilbene found in blueberries and Pterocarpus marsupium heartwood is structurally similar to resveratrol. It is used for the treatment of cancer, diabetes and fungal infections. Three spectrophotometric methods have been developed for the determination of Pterostilbene. Two first derivative methods and one difference spectrophotometric method were attempted. Two first derivative spectroscopic methods were developed in sodium hydroxide (Method A) and methanol (Method B) and differential spectroscopic method (Method C) was developed using borate buffer and sodium hydroxide. Linearity was observed over the concentration range 0.1-25 and 1-25 µg/ml with linear regression equations y = 0.0043x-0.0003 ($R^2$=0.9997) and y=0.0037x - 0.0007 ($R^2$=0.9997) for method A and B respectively whereas for method C the linearity was followed over the concentration range 0.1-20 µg/ml with linear regression equation y=0.1124x - 0.0016 (R2=0.9999). The three methods were validated and can be applied for the determination of Pterostilbene pharmaceutical formulations.

**Key words:** Derivative spectroscopy, Differential spectroscopy, Pterostilbene, Validation, Borate buffer, Methanol, Sodium Hydroxide.

**Correspondence:**
Mathrusri Annapurna Mukthinuthalapati,
Department of Pharmaceutical Analysis,
GITAM Institute of Pharmacy,
GITAM University, Visakhapatnam,
India-530045.

Fax: 91-891-2795315
Mobile no: 91-9985654603

E-mail:- mathrusri2000@yahoo.com
**DOI:** 10.5530/phm.2015.6.22

## INTRODUCTION

Pterostilbene (PTS) (Figure 1) belongs to the group of phytoalexins produced by plant[1] also found in red wine is a dimethylated analogue of resveratrol. It is found in blueberries, grapes and in age-old darakchasava, an ayurvedic medicine from India[2] and in the tree species Pterocarpus marsupium and Guibourtia tessmanii.[3-8] It is believed to exhibit anti-cancer activity,[9-11] anti-fungal activity[12] anti-diabetic and antioxidant actions.[13-6]

Three liquid chromatographic methods were developed for the determination of Pterostilbene in rat plasma with fluorescence detection[17] dragon blood[18] and rat plasma in gradient mode[19] and no spectrophotometric method has been developed for the assay of Pterostilbene in pharmaceutical formulations. Currently the authors have proposed three simple, rapid, precise and robust validated spectrophotometric methods (derivative and difference spectroscopy) for the determination of PTS in capsules.

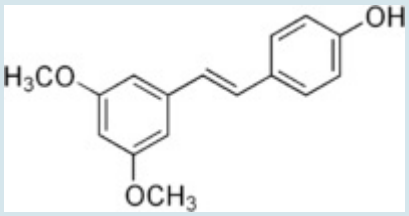



**Figure 1:** Structure of Pterostilbene

## MATERIALS AND METHODS

### *Chemicals and Reagents*

Pterostilbene standard (>99.0% purity) was obtained from Oxford laboratory, India. Sodium hydroxide, methanol and boric acid were obtained from Merck (India). All chemicals were of analytical grade and used as received. Pterostilbene is available as capsules with brand names such as PTEROSTILBENE (Source Naturals Inc. (Canada); Label claim: 50 mg) (Brand I), PTEROSTILBENE (Absorb Health (North Carolina); Label claim: 100 mg) (Brand II). Pterostilbene is procured from Booyahchicago, Delhi, India.

### *Instrumentation*

A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu) loaded with spectra manager software UV Probe was employed with spectral band width of 1 nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. 400 nm to 200 nm the wavelength range was selected for scanning with medium scanning speed.

### *Procedure*

### *Preparation of Pterostilbene stock and working standard solutions*

Pterostilbene stock solution (1000 µg/ml) was prepared by dissolving 25 mg of PTS in methanol in a 25 ml volumetric flask. Working solutions were prepared from the stock solution with on dilution with sodium hydroxide, methanol and borate buffer as per the requirement for method A, B and C respectively.

### *Preparation of borate buffer (pH-9.0)*

6.20gm of boric acid was dissolved in 500ml of distilled water, adjusted to pH 9.0 with 0.1 M sodium hydroxide (about 41.5ml) and diluted to 1000ml with distilled water.

### *Derivative spectroscopic methods (Method A and Method B)*

A series of Pterostilbene solutions 0.1-25 µg/ml and 1-25 µg/ml were prepared from the stock solution by diluting with 0.1 N NaOH and methanol respectively and scanned (200-400 nm) for method A and method B. The resulting zero order absorption spectra obtained were converted in to first derivative spectra by the inbuilt software of the instrument.

### *Differential spectroscopic method (Method C)*

A series of Pterostilbene solutions 0.1-20 µg/ml were prepared from the stock solution by diluting with 0.1 N NaOH and borate buffer separately. The solutions prepared in sodium hydroxide were used in reference

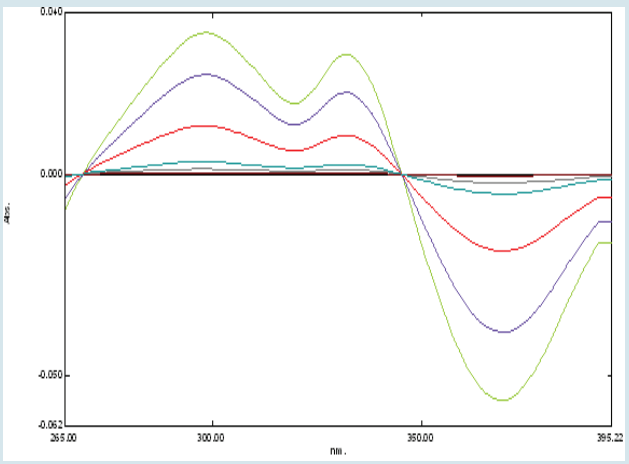


**Figure 2:** Overlay Derivative Spectrum of Pterostilbene in Sodium hydroxide (Method A)

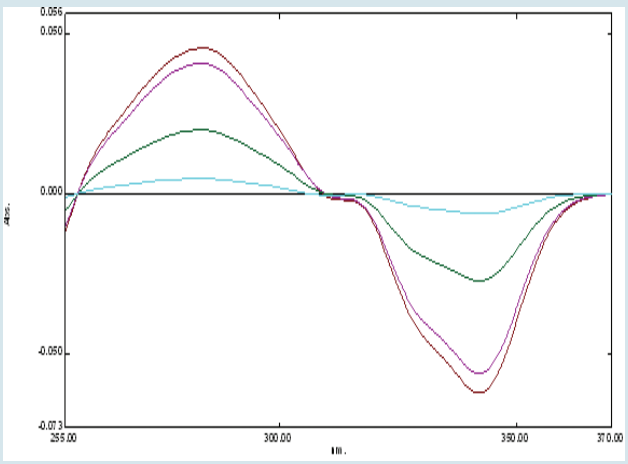


**Figure 3:** Overlay Derivative spectrum of Pterostilbene in Methanol (Method B)

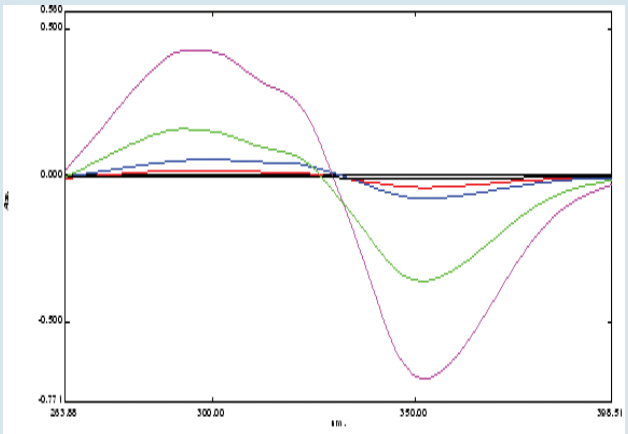


**Figure 4:** Overlay Difference spectrum of Pterostilbene (Method C)

**Table 1: Linearity of Pterostilbene**

| Conc. (µg/ml) | Absorbance | | | | |
|---|---|---|---|---|---|
| | Method A | Method B | Method C | | |
| | | | Maxima | Minima | Amplitude |
| 0.1 | 0 | - | 0.004 | 0.012 | 0.016 |
| 0.5 | 0.002 | - | 0.016 | 0.039 | 0.055 |
| 1 | 0.005 | 0.004 | 0.004 | 0.078 | 0.118 |
| 5 | 0.02 | 0.02 | 0.02 | 0.358 | 0.568 |
| 10 | 0.042 | 0.038 | 0.038 | 0.7 | 1.12 |
| 15 | 0.065 | 0.055 | 0.055 | 1.048 | 1.678 |
| 20 | 0.086 | 0.074 | 0.074 | 1.414 | 2.264 |
| 25 | 0.108 | 0.092 | 0.092 | - | - |

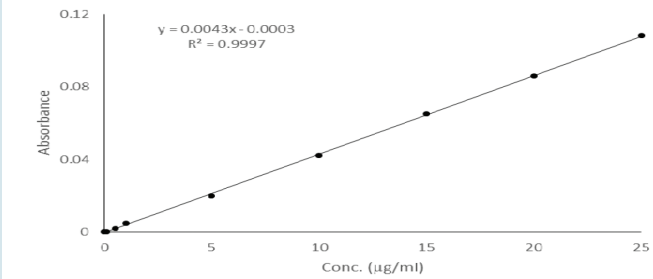
**Table 2: Optical characteristics of Pterostilbene**

| Characteristics | Method A | Method B | Method C |
|---|---|---|---|
| λ (nm) | 369 (Minima) | 283 (maxima) | 294-353 (Amplitude) |
| Linearity (µg/ml) | 0.1-25 | 1-25 | 0.1-22 |
| Regression equation | 0.0043x-0.0003 | 0.0037x-0.0007 | 0.1124x - 0.0016 |
| Slope | 0.0043 | 0.0037 | 0.1124 |
| Intercept | 0.0003 | 0.0007 | 0.0016 |
| $R^2$ | 0.9997 | 0.9997 | 0.9999 |

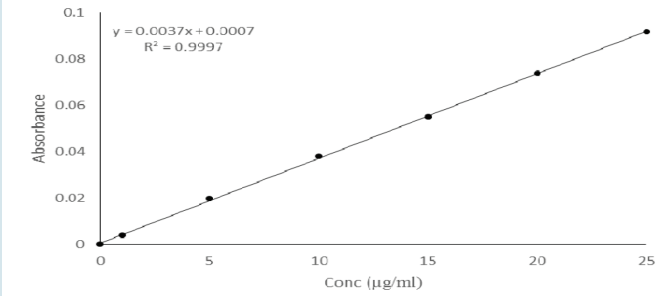
**Table 3: Assay of Pterostilbene marketed formulation (Capsules)**

| Formulation | Method | | | % Recovery * | | |
|---|---|---|---|---|---|---|
| | A | B | C | A | B | C |
| Brand I | 49.25 | 49.22 | 49.58 | 98.50 | 98.45 | 99.16 |
| Brand II | 98.73 | 99.4 | 98.95 | 98.73 | 99.40 | 98.95 |

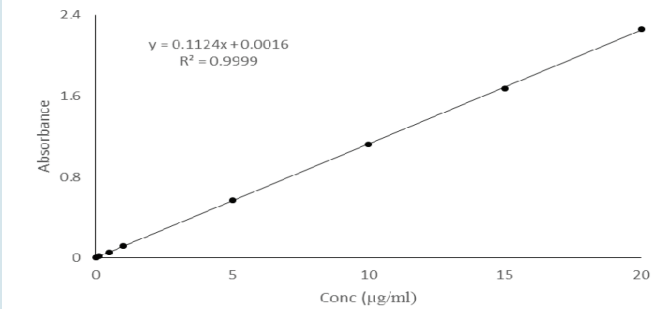
*Mean of three replicates.




[A]




[B]




[C]

**Figure 5:** Calibration curve of Pterostilbene in method [A], [B] and [C]

cuvette as blank and the same concentration solutions prepared in borate buffer were scanned by keeping them in the sample cuvette for method C.

### *Validation*[20]

#### *Linearity*

#### *Method A*

First derivative spectrophotometric method was developed in sodium hydroxide solution for the determination of PTS. The zero order absorption spectra so obtained (0.1-25 µg/ml) was converted in to first derivative spectra by the inbuilt software of the instrument. The resulting derivative spectra show both maxima and minima at 332 nm and 369 nm but the magnitude of minima was selected for all the analytical calculations. A calibration curve was drawn by plotting the concentration of the drug solution on the x-axis and the corresponding minima values on the y-axis.

#### *Method B*

The first derivative spectra obtained for Pterostilbene drug solutions (1-25 µg/ml) show both maxima and minima at 283 nm and 342 nm but the maxima was selected for all the analytical calculations. A calibration curve was drawn by plotting the concentration of the drug solution on the x-axis and the corresponding maxima values on the y-axis.

#### *Method C*

A series of Pterostilbene drug solutions (0.1-22 µg/ml) were prepared in sodium hydroxide and scanned (200-400 nm) against the solutions prepared in borate buffer of the same concentration and the difference absorption spectra was recorded. The difference absorption spectrum has shown minima at 353 nm and maxima at 294 nm and therefore the amplitude was selected for all the analytical calculations. A graph was drawn by plotting the concentration of the drug solutions on the x-axis and the corresponding amplitude values on y-axis.

#### *Precision and accuracy*

The intra-day and inter-day precision studies of the three methods were performed at three different concentration levels (10, 15 and 20 µg/ml) and on three different days respectively and the % RSD was calculated. The accuracy of the assay method was calculated at three different levels (80%, 100% and 120%) by the standard addition method and the percentage recoveries were calculated for method A, B and C with sodium hydroxide, methanol and borate buffer respectively.

#### *Assay of marketed formulations (Capsules)*

Twenty capsules of Pterostilbene were procured from the local pharmacy store and the contents were finely powdered and powder equivalent to 100 mg PTS was accurately weighed and dissolved in sodium hydroxide, methanol and borate buffer in a 100 ml volumetric flask separately and the contents of the flask were sonicated for 30 min and filtered for method A, B and C respectively. These solutions were diluted as per the requirement with the respective solvents and analysed.

## RESULTS AND DISCUSSION

Three new analytical methods (two first derivative and one difference spectrophotometric method) have been developed for the determination of Pterostilbene using sodium hydroxide (Figure 2), methanol (Figure 3) and borate buffer-sodium hydroxide (Figure 4) for method A, B and C respectively. The minima, maxima and the amplitude were chosen for all the analytical calculations for method A, B and C respectively from the respective spectra. Pterostilbene shows linearity over the concentration range 0.1-25, 1-25 and 0.1-20 µg/ml (Table 1) with linear regression equations y=0.0043x - 0.0003 ($R^2$=0.9997), y=0.0037x - 0.0007 ($R^2$=0.9997) and y=0.1124x - 0.0016 ($R^2$=0.9999) for method A, B and C respectively (Figure 5). The optical characteristics of the three methods A, B and C were shown in Table 2.

#### *Assay of marketed formulations (Capsules)*

The proposed three analytical techniques were applied for the assay of Pterostilbene in capsules (PTEROSTILBENE® Source Naturals Inc.; Label claim: 50 mg and PTEROSTILBENE® Absorb Health.; Label claim: 100 mg) and the percentage of recovery is found to be 98.50-98.73, 98.45-99.40 and 98.95-99.16 for method, B and C (Table 3) respectively.

## CONCLUSION

The proposed validated derivative and differential spectrophotometric methods are simple, precise, accurate and can be applied for the determination of Pterostilbene in pharmaceutical formulations successfully.

## ACKNOWLEDGMENTS

The authors are grateful to M/s GITAM University, Visakhapatnam for providing the research facilities. The authors have no conflict of interest.

## ABBREVIATION USED

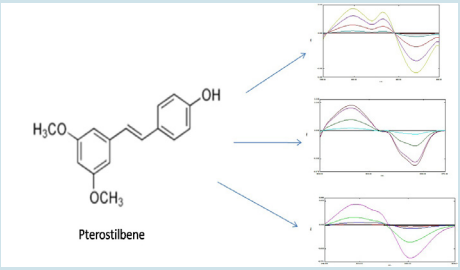
**PTS:** Pterostilbene; **LC-MS:** Liquid Chromatography-Mass Spectroscopy; **UPLC:** Ultra Pressure Liquid Chromatography; **HPLC:** High Performance; **HPTLC:** High Pressure Thin Layer Chromatography; **UV:** Ultraviolet; **RSD:** Relative Standard Deviation; **ICH:** International Conference on Harmonization.

## REFERENCES

1. Langcake P, Pryce RJ. A new class of phytoalexins from grapevines. Experientia. 1977;33(2):151-2.
2. Bernard P, Isaac M, Jayant D, and Claudine C. Occurrence of Resveratrol and Pterostilbene in age-old darakchasava, an ayurvedic medicine from India. Journal of Ethnopharmacology. 1999;68(1):71-6.
3. Fuendjiep V, Wandji J, Tillequin F, Mulholland DA, Budzikiewicz H, Fomum ZT, Nyemba AM, Kock M. Chalconoid and stilbenoid glycosides from Guibourtia tessmanii. Phytochemistry. 2002;60(8):803-6.
4. Rimando M, Wilhelmina K, Magee JB, Dewey J, Ballington JR, Agric J. Resveratrol, Pterostilbene and piceatannol in vaccinium berries. Food Chem. 2004; 52(15):4713-9.
5. Pezet R, Pont V. Identification of Pterostilbene in grape berries of Vitis vinifera. Plant Physiology and Biochemistry. 1988;26(5):603-7.
6. Manickam M, Ramanathan M, Farboodniay Jahromi MA, Chan-souria JPN, Ray AB. Antihyperglycemic activity of phenolics from Pterocarpus marsupium. Journal of Natural Products. 1997;60(6):609-10.
7. Adrian M, Jeandet P, Douillet-Breuil AC, Tesson L, Bessis R. Stilbene content of mature vitis vinifera berries in response to UV-C elicitation. Journal of Agricultural and Food Chemistry. 2000; 48: 6103-05.
8. Douillet-Breuil AC, Jeandet P, Adrian M, Bessis R. Changes in the phytoalexin content of various vitis spp in response to ultraviolet C elicitation. Journal of Agricultural and Food Chemistry. 1999;47:4456-61.
9. Roberti M, Pizzirani D, Simoni D, Rondanin R, Baruchello R, Bonora C, Buscemi F, Grimaudo S, Tolomeo M. Synthesis and biological evaluation of resveratrol and analogues as apoptosis-inducing agents. Journal of Medicinal Chemistry. 2003;46(16):3546-54.
10. Tolomeo M, Grimaudo S, Cristina AD, Roberti M, Pizzirani D, Meli M, Dusonchet L, Gebbia N, Abbadessa V, Crosta L, Barucchello R, Grisolia G, Invidiata F, Simoni D. Pterostilbene and 3′-hydroxyPterostilbene are effective apoptosis-inducing agents in MDR and BCR-ABL-expressing leukemia cells. The International Journal of Biochemistry & Cell Biology. 2005;37(8):1709-26.
11. Ferrer P, Asensi M, Segarra R, Ortega A, Beniloch M, Obrador E, Varea MT, Asensio G. Jorda L, Estrela JM. Association between Pterostilbene and quer-

cetin inhibits metastatic activity of B16 melanoma; Neoplasia. 2005;7(1):37-47.
12. Jeandet P, Douillet-Breuil AC, Bessis R, Debord S, Sbaghi M, Adrian M. Phytoalexins from the vitaceae biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. Journal of Agricultural and Food Chemistry. 2002;50(10):2731-41.
13. Stivala LA, Savio M, Carafoli F, Perucca P, Bianchi L, Maga G, Forti L, Pagnoni UM, Albini A, Prosperi E, Vannini V. Specific structural determinants are responsible for the antioxidant activity and the cell cycle effects of resveratrol. Journal of Biological Chemistry. 2001;276(25):22586-294.
14. Rimando AM, Cuendet M, Desmarchelier C, Mehta RG, Pezzuto JM, Duke SO. Cancer chemopreventive and antioxidant activities of pterostilbene, a naturally occurring analogue of resveratrol. Journal of Agricultural and Food Chemistry. 2002;50(12):3453-7.
15. Amorati R, Lucarini M, Mugnaini V, Pedulli GF. Antioxidant Activity of Hydroxystilbene Derivatives in homogeneous solution. The Journal of Organic Chemistry. 2004;69(21):7101-7.
16. Akansha M, Rohit S, Swayam PS, Sudeep G, Rakesh M, Akhilesh KT, Arvind KS. Confirmation towards establishing antidiabetic activity in heart wood of pterocarpus marsupium and analysis of phytoconstituents. Indian Journal of Experimental Biology. 2013;51:363-74.
17. Connie MR, Jaime AY, Kathryn AR, Neal MD. High-performance liquid chromatographic analysis of Pterostilbene in biological fluids using fluorescence detection. Journal of Pharmaceutical and Biomedical Analysis. 2007;43(1):250-4.
18. Ying-qing HU, Ning Z, Dai-lin LIU. RP-HPLC studies on quantitative determination of Pterostilbene in dragon's blood. Chinese Journal of Pharmaceutical Analysis. 2002;22(6):428-30.
19. Hai-Shu L, Bing-De Y, Paul CH. Determination of Pterostilbene in rat plasma by a simple HPLC-UV method and its application in pre-clinical pharmacokinetic study. Biomedical Chromatography. 2009;23(12):1308-15.
20. ICH. Validation of analytical procedures: Text and methodology Q2 (R1). International Conference on Harmonization; 2005.

## PICTORIAL ABSTRACT




## SUMMARY

- Two simple, precise and accurate derivative and differential spectrophotometric methods were developed for the determination of Pterostilbene and validated.

## ABOUT AUTHOR

**Mathrusri Annapurna Mukthinuthalapati:** Obtained her Ph. D. degree from Berhampur University, Berhampur, Orissa. Currently, she is working as Professor at GITAM Institute of Pharmacy, GITAM University, Visakhapatnam, India. Dr. Mathrusri Annapurna is working on analytical method development, validation, forced degradation studies of drug molecules and also on computer augmented simulated studies of metal complexes of drug molecules.