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Novel Second Derivative Spectrophotometric Methods for the Quantification of Pterostilbene (An Anti- Diabetic Agent)

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ABSTRACT

Pterostilbene (trans-3,5-dimethoxy-4-hydroxystilbene) is a natural dietary compound and the primary antioxidant component of blueberries and and Pterocarpus marsupium heartwood. Substantial evidence suggests that pterostilbene may have numerous preventive and therapeutic properties in a vast range of human diseases that include neurological, cardiovascular, metabolic, and hematologic disorders. Four new second derivative spectrophotometric methods have been developed for the determination of Pterostilbene in pharmaceutical dosage forms. The authors have developed four second derivative spectrophotometric methods in borate buffer, methanol, sodium hydroxide and phosphate buffer. The proposed spectrophotometric methods were validated and can be applied for the determination of Pterostilbene in pharmaceutical formulations (Capsules).

KEY WORDS: Pterostilbene, Phosphate buffer, Borate buffer, Sodium hydroxide, Methanol, Derivative spectroscopy, Validation.

1. INTRODUCTION

Pterostilbene (PTS) (Figure 1) is found in blueberries, grapes and in age-old darakchasava, an ayurvedic medicine from India (Bernard, 1999) and in the tree species Pterocarpus marsupium and Guibourtia tessmanii (Fuendjiep, 2002; Rimando, 2004; Pezet, 1988; Manickam, 1997; Adrian, 2000 and Douillet-Breuil, 1999). It is believed to exhibit anti-cancer activity (Roberti, 2003; Tolomeo, 2005 and Ferrer, 2005), anti-fungal activity (Jeandet, 2002) anti-diabetic and antioxidant actions (Stivala, 2001; Rimando, 2002; Amorati, 2004; Akansha, 2013). Chemically it is trans 3, 5-dimethoxy-4'-hydroxy-trans-stilbene, a group of phytoalexins produced by plant (Langcake, 1977). It is a dimethylated analogue of resveratrol, $C_{16}H_{16}O_3$ with molecular weight 256.296 g/mol. Three chromatographic methods were developed for the estimation of Pterostilbene with fluorescence detection (Connie, 2007) in dragon blood (Ying-qing, 2002) and rat plasma in gradient mode (Hai-Shu, 2009) and no spectrophotometric method has been developed till now for the determination of pharmaceutical formulations. In the present study the authors have proposed four simple, rapid, precise and robust validated spectrophotometric methods (derivative spectroscopy) for the determination of PTS.

2. MATERIALS AND METHODS

2.1. Instrumentation: A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe was employed with spectral bandwidth of 1nm and wavelength accuracy of ± 0.3 nm with a pair of 10 mm matched quartz cells. All weights were taken on electronic balance (Shimadzu).

2.2 Chemicals and reagents: Analytical grade methanol (Merck), disodium phosphate (Na_2HPO_4) (Merck), mono potassium phosphate (KH_2PO_4) (Merck), boric Acid, sodium hydroxide, methanol, glacial acetic acid were used. Pterostilbene is available as capsules with brand names such as Pterostilbene (Source Naturals Inc. (Canada), Label claim: 50 mg), Pterostilbene (Absorb Health (North Carolina), Label claim: 100 mg). Pterostilbene is procured from Booyahchicago, Delhi, India.

2.3. Preparation of phosphate buffer pH 4.0: 5.04 grams of disodium hydrogen phosphate and 3.01 grams of potassium dihydrogen phosphate were added in sufficient water to produce 1000ml and adjust the pH to 4.0 with glacial acetic acid.

2.4. Borate buffer pH 9.0: 6.20 g of boric acid was dissolved in 500 mL volumetric flask with water. This solution was transferred into a 1000ml volumetric flask along with 41.5 mL of 1M sodium hydroxide and diluted with water to 1000 mL to adjust pH 9.0.

2.5. Preparation of stock and sample solutions: The standard solution of Pterostilbene was prepared by dissolving accurately about 100 mg of the Pterostilbene in methanol in a 100 mL volumetric flask. The stock solution was further diluted with borate buffer, methanol, sodium hydroxide, and phosphate buffer as per the requirement for method A, B, C and D respectively.

2.6. Procedure for preparation of calibration curve: A series of Pterostilbene solutions 1-25, 1-20, 0.1-15 and 1-25 µg/mL were prepared in borate buffer, methanol, sodium hydroxide and phosphate buffer for method A, B, C and D respectively and scanned (200-400 nm) against their reagent blank. The detection of absorption maxima i.e. λmax was difficult to identify in the zero absorption spectra so obtained for method A, B, C and D and therefore

the spectra were converted in to second derivative spectra by the inbuilt software of the instrument for further investigation. The maxima was chosen from the second derivative spectra for further analytical calculations for method A, C and D whereas the amplitude was selected for method B. A graph was drawn by taking the concentration of the drug solutions on the x-axis and the corresponding derivative absorbance on the y-axis for all the methods.

2.7. Assay of marketed formulations of Pterostilbene (capsules): Pterostilbene is available with brand names PTEROSTILBENE (Source Naturals Inc. (Canada), Label claim: 50 mg), PTEROSTILBENE (Absorb Health (North Carolina), Label claim: 100 mg) as capsules. To perform the assay of QC samples of Pterostilbene twenty capsules were procured from the local pharmacy store and the contents were extracted with borate buffer, methanol, sodium hydroxide and phosphate buffers for method A, B and C and D respectively and the dilutions were made from this stock as per the requirement and the percentage recovery was calculated.

2.8. Precision and accuracy: Precision study was performed by taking the derivative absorbance of six replicates (20 µg/mL) were measured for Method A, B, C and D and the % RSD was calculated. Accuracy of the method was evaluated by the percent recovery studies. This study was performed by adding 80%, 100%, and 120% of pure drug solutions to a constant concentration of extracted formulation (pre-analysed formulation) solution and the % RSD was calculated.

3. RESULTS AND DISCUSSION

Four new second derivative spectrophotometric methods were developed for the determination of Pterostilbene in capsules in borate buffer, methanol, sodium hydroxide and phosphate buffer. The resulting derivative spectra were shown in Figure 2, 3, 4 and 5. For method A, C and D the maxima was chosen from the derivative spectra for the linearity study. The derivative absorbance was noted at 284.67, 325.17 and 349.06 nm and the absorbance was plotted against concentration for method A, B and D respectively for the construction of the calibration curve. In method B Pterostilbene has shown maxima at 229.04 and minima at 238.08 nm and therefore the amplitude was selected for further calculations. Also the zero crossing points were observed at 226.02, 232.62, 244.27, 284.27 and 342.52 nm indicating the λmax of the drug.

Beer's law was obeyed over the concentration range 1-25 µg/mL for methods A and D with regression equations y = 0.0025x + 0.0003 and y = 0.0003x + 0.0000007 respectively. Pterostilbene also obeys Beer Lambert's law 1-20 and 0.1-15 µg /mL with regression equations y = 0.0004x + 0.000008 and y = 0.0001x + 0.00001 respectively for method B and C respectively. The calibration curves obtained were shown in Figure 6A-6D.

The % RSD in precision studies were found to be 0.33, 0.42, 0.28 and 0.31 for method A, B C and D respectively (RSD <2%) indicating that the method is precise. The % RSD in accuracy studies were found to be 0.143, 0.221, 0.321 and 0.442 for method A, B, C and D respectively (RSD <2%) indicating that the method is more accurate.

The marketed formulations were evaluated by the proposed methods and the percentage recovery was calculated (Table 1). The percentage recovery was found to be 99.02-99.32, 98.48-98.89, 98.96-99.45 and 99.46-99.49 for method A, B C and D respectively.

Table.1. Assay of marketed formulations of Pterostilbene (Capsules)

Brand	Labeled Amount (mg)	*Amount obtained (mg)				% Recovery*			
		Method				Method			
		A	B	C	D	A	B	C	D
I	50	49.66	49.24	49.48	49.73	99.32	98.48	98.96	99.46
II	100	99.02	98.89	99.45	99.46	99.02	98.89	99.45	99.46

*Each value is average of three determinations

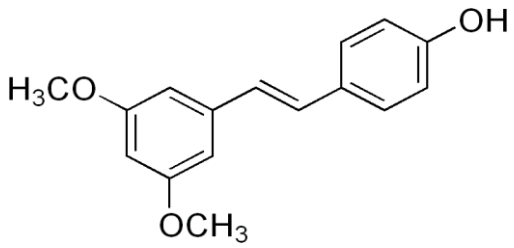

Figure. 1. Chemical structure of Pterostilbene (PTS)

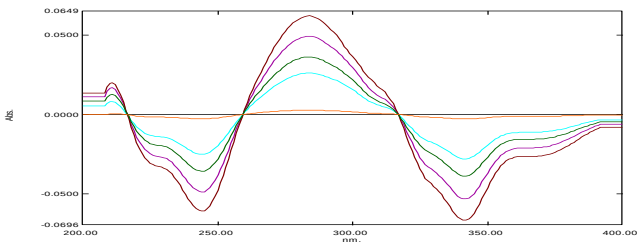

Figure. 2. Overlay second derivative spectrum of Pterostilbene in borate buffer

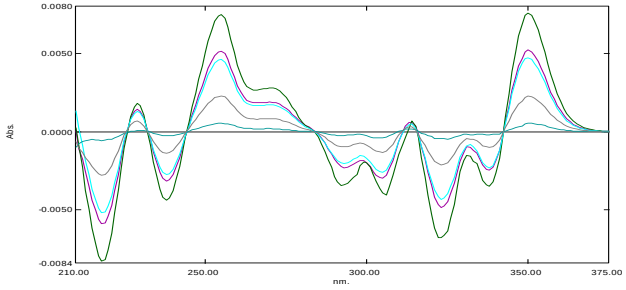

Figure. 3. Overlay second derivative spectrum of Pterostilbene in methanol

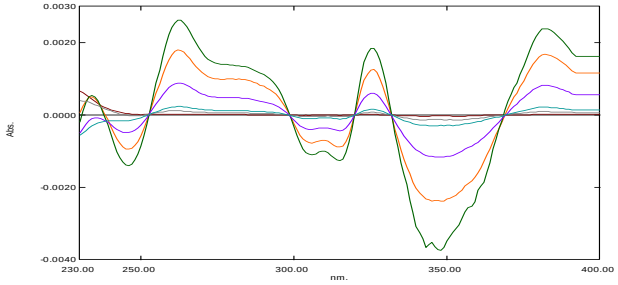

Figure. 4. Overlay second derivative spectrum of Pterostilbene in sodium hydroxide

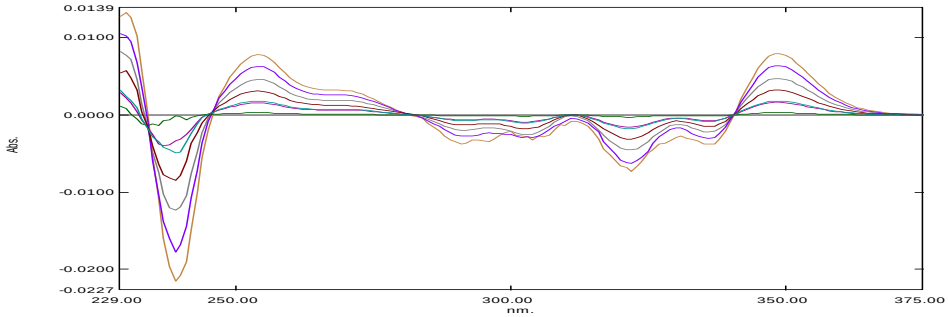

Figure. 5. Overlay second derivative spectrum of Pterostilbene in phosphate buffer

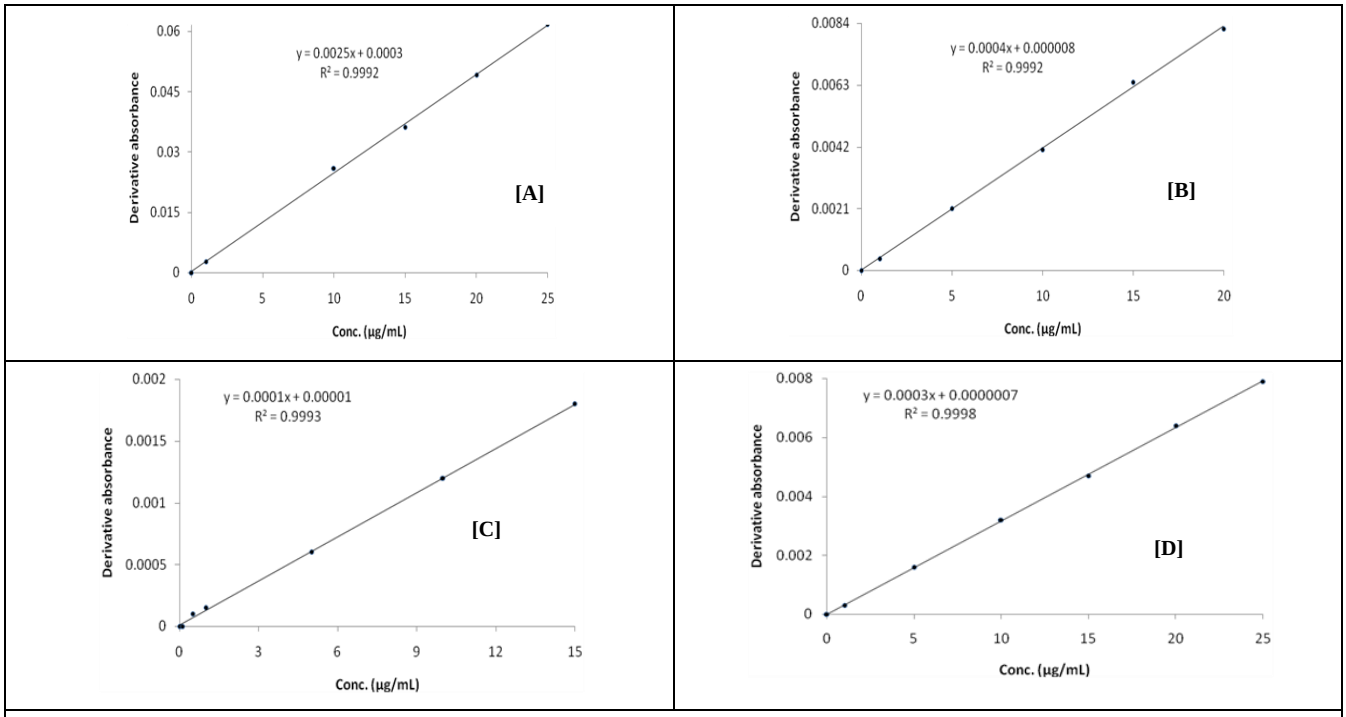

Figure. 6. Calibration curves of Pterostilbene in A) Borate buffer B) Methanol C) Sodium hydroxide and D) Phosphate buffer

4. CONCLUSION

The second derivative spectrophotometric techniques were developed for the determination of Pterostilbene validated and can be applied for the pharmaceutical formulations successfully.

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