Original Article

Development of a Validated Stability Indicating Liquid Chromatographic Method for the Determination of Pterostilbene

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ABSTRACT

Introduction: Pterostilbene, a stilbenoid possess diversified pharmacological activities. In the present study, a stability-indicating liquid chromatographic method was proposed for the determination of Pterostilbene in pharmaceutically available formulations. **Materials and Methods:** The chromatographic separation was achieved on Phenomenex C8 type column as stationary phase. UV detection was carried out at 219 nm. Pterostilbene was subjected to various stress conditions such as acidic, alkaline, oxidative, thermal and photolytic degradations. **Results:**The drug was found to be sensitive towards acidic and alkaline stress conditions. The proposed method was validated as per the ICH guidelines and successfully applied to the available marketed formulations. **Conclusion:** The proposed method is selective, specific, robust and can be applied for the assay of pharmaceutical dosage forms.

Key words: Pterostilbene, Stability-Indicating, RP-HPLC, Validation, ICH guidelines.

INTRODUCTION

Pterostilbene (PTB), was first isolated from *Pterocarpus santalinus* (red sandalwood),[1] and is an active constituent in *Pterocarpus marsupium*.[2-5] It is traditionally used as the medicine in the therapy of diabetes. It has also shown diversified pharmacological activities: anti hyper-glycemic;[6] hypolididemic;[7] anti-cancer;[8-10] anti-diabetic[11-13] and anti-fungal[14] properties. PTB, a methyl ether of resveratrol, which is chemically 3,5-dimethoxy-4'- hydroxy-trans-stilbene ($C_{16}H_{16}O_3$); Mol. Wt. – 256.296 g/mol (Figure 1).

Only two liquid chromatographic methods have been developed for the determination of Pterostilbene, one with fluorescence detection[15] and the other in rat plasma[16] and also one spectrophotometric method.[17] Till date from the literature available it is evident that no stability indicating liquid chromatographic method was available assay of Pterostilbene in formulations.

MATERIALS AND METHODS

Instrumentation and Chromatographic Conditions

The chromatographic separation was successfully achieved on - HPLC system: UFLC Shimadzu Prominence system (CBM-20Alite) model equipped with SPD M20A detector (PDA); Stationary Phase: Phenomenex C8 type column (250 mm × 4.6 mm i.d., 5 µm particle size); Mobile Phase: 0.1% Trifluoroacetic acid (TFA) in water (v/v): acetonitrile (10:90 %, v/v); Flow rate: 0.6 ml/min; Injection volume: 20 µl; UV detection wavelength: 219 nm; Temperature: Ambient (25°C).

Chemicals and Reagents

Pterostilbene standard (>99.0% purity) was obtained from Oxford laboratory, India. It is available as capsules with brand names: PTEROSTILBENE (Source Naturals Inc. (Canada); Label claim: 50 mg), PTEROSTILBENE (Absorb Health (North Caro-

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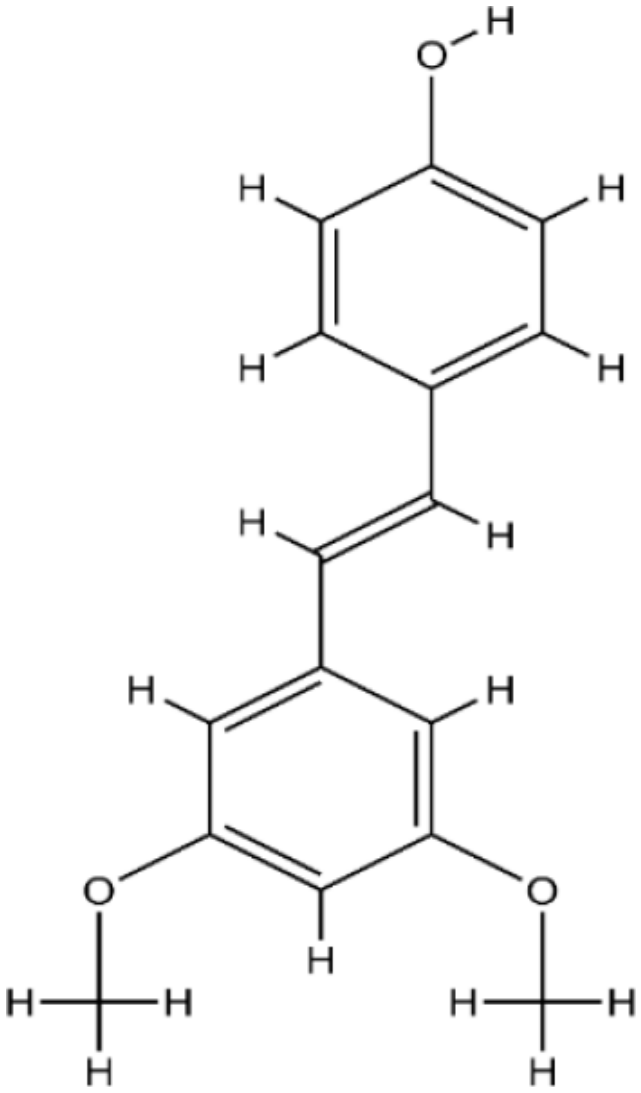

Figure 1: Structure of Pterostilbene.

lina); Label claim: 100 mg). Trifluoroacetic acid (TFA), Water, Methanol, Sodium hydroxide (NaOH), Hydrochloric acid (HCl) and Hydrogen peroxide (H_2O_2) were purchased from Merck (India) and all chemicals are of HPLC grade and used as received.

Preparation of 0.1% TFA Buffer (v/v)

The mobile phase was prepared by accurately transferring 1ml of TFA in to a 1000 ml volumetric flask and dissolved with HPLC grade water.

Preparation of Stock Solution

The stock solution was prepared by transferring accurately 25 mg of PTB in to a 25 ml volumetric flask and diluting with mobile phase (1000 µg/ml) and further dilutions were made on daily basis from the stock solution with mobile phase as per the requirement.

Validation

The developed method was validated as per ICH Q2(R1) guidelines, 2005.[18] The linearity (0.02–250 µg/ml) and precision (10, 20 and 50 µg/ml) studies were conducted. The accuracy studies were accomplished with three pre-delineate spiked concentration levels (80, 100 and 120%). In robustness study the chromatographic conditions were marginally modified for: flow rate (0.54 and 0.66 ml/min); % organic phase (88 and 92% v/v) and detection wavelength (217 and 221 nm). The robustness study was carried at a concentration level of 20 µg/ml of the standard. All the validation data obtained was taken in triplicate.

Forced Degradation Studies

To confirm that the analytical method is stability-indicating, PTB was exposed to stress under to various conditions to accomplish forced degradation studies.[19] As the present ICH guidelines did not make any statement regarding the detailed degradation conditions in the stress testing, the presently used forced degradation conditions were all based on trial and error.

Acidic degradation was performed by refluxing stock solution (1ml) with 1 ml of 1N HCl for a time of 60 min at 80℃ in a thermostat, the solution was cooled, neutralized and then diluted as per requirement. Alkaline degradation was conducted by heating stock solution (1ml) along with 1 ml of 1N NaOH for a period of 60 min at 80℃, later it was cooled, neutralized and then diluted as per requirement. During oxidative degradation, stock solution (1ml) was left in a 10ml volumetric flask with 1.0 ml of 30% H_2O_2 for a period of 60 min at 40℃, later cooled and then diluted. Thermal degradation was conducted by exposing a definite quantity of PTB standard in solid state and also in solution state to dry heat in a hot air oven maintained at 80 ºC continuously for 7 days and the resultants were cooled and diluted before the study. For Photolytic degradation, the sample in solid state taken in a petri-plate and placed in a UV cabinet for the exposure to cool florescent and near UV lamp continuously for about 7 days. The sample was spread as a thin layer and sealed with a transparent cover to minimize the effects of the changes in the physical state. . The solution was prepared with acetonitrile and further dilution with diluent.

Assay of Marketed Formulations

Twenty capsules of each brand of PTB were procured from the local pharmacy store, weighed, crushed into fine powder and was extracted and filtered, from which

Table 1: Comparison of Previously Published Methods for Pterostilbene with Present Method.

Mobile phase/Reagent	λ (nm)	Linearity (µg/ml)	Method	Reference
Acetonitrile: water (50:50, v/v)	Excitation 330 Emission 374	5-100	HPLC	15
Acetonitrile: 0.1% formic acid	320	0.2-20	HPLC	16
0.1% TFA: Acetonitrile (10:90)	219	0.02-250	Stability indicating RP-HPLC	Present work

the final dilutions were made as per the requirement prior analysis.

RESULTS AND DISCUSSION

Method Development and Optimization

An attempt has been made to develop a stability indicating RP-HPLC method for the assay of PTB in pharmaceutical products. From the literature, it was evident that only two chromatographic methods were available, which were summarized in comparative study given in Table 1 and compared with the present method. PTB in its UV spectrum (Figure 2), shows maximum absorbance at 219 and 307 nm, but the degradations products formed were clearly detect only at 219 nm, for which it was selected as the wavelength of detection. The drug samples were analyzed using different mobile phase compositions and flow rates. 0.1% TFA in water (v/v) is adopted as aqueous phase, as it suppresses the ionization of residual silinols present in the stationary phase and thereby facilitates sharp peaks with low tailing factor. Finally, a mixture of 0.1% TFA in water (v/v): acetonitrile (10:90%, v/v) with a flow rate of 0.6 ml/min has produced a desirable sharp peak with suitable system suitability parameters. The typical chromatograms of blank and PTB was shown in Figure 3a-3b respectively.

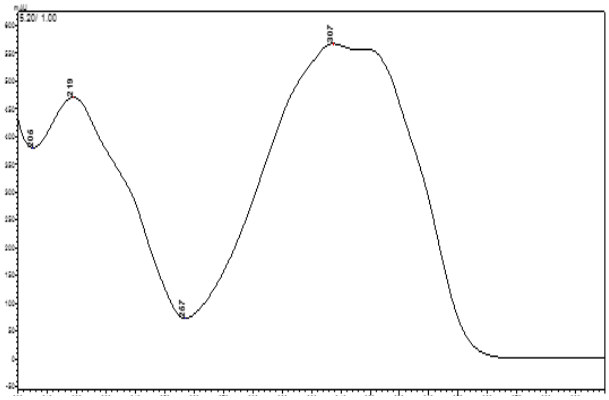

Figure 2: UV Spectrum of Pterostilbene.

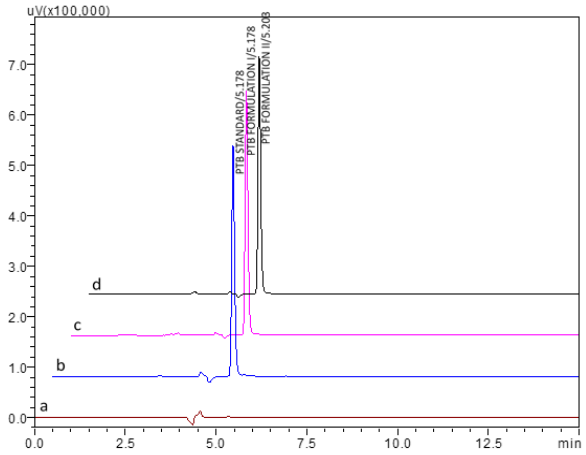

Figure 3: Typical Chromatograms of: (a) Blank; (b) Pterostilbene standard (20 µg/ml); (c) Formulation I (20 µg/ml) and (d) Formulation II (20 µg/ml)

Method Validation

The method was validated for system suitability, linearity, limit of quantitation (LOQ), limit of detection (LOD), precision, accuracy, selectivity and robustness.[18]

Linearity

PTB shows linearity over a concentration range of 0.02–250 µg/ml, with their percentage relative standard deviations (%RSD) in limits (Table 2). The limit of quantitation (LOQ), limit of detection (LOD) were found to be 0.01965 and 0.00648 µg/ml respectively.

Accuracy

The method accuracy was demonstrated by the % recovery at three different concentrations i.e., 36, 40 and 44 µg/ml (80, 100 and 120%), in which the known amount of standard was spiked to the samples. The % recovery was found to be 98.91-99.59% and %RSD in limit with and very low standard error mean (SEM).

Precision

Precision studies were conducted at three concentrations i.e., 10,20, 50 µg/ml for both intra-day and inter-day precision for which the %RSD was found to be 0.02-0.67% (Table 3).

Robustness

Table 2: Linearity of Pterostilbene.		
Concentration (µg/ml)	***Mean Peak Area ± SD**	**RSD (%)**
0.02	3425 ± 10.96	0.32
0.5	85138 ± 127.71	0.15
1	170349.09 ± 545.12	0.32
2	342598 ± 1438.91	0.42
5	852099 ± 937.31	0.11
10	1701798 ± 1361.44	0.08
15	2577209 ± 9793.39	0.38
20	3409584 ± 15343.13	0.45
50	8513960 ± 23839.09	0.28
100	17909796 ± 66266.25	0.37
200	34095870 ± 61372.57	0.18
250	42618800 ± 268498.44	0.63
Slope ± SD	: 175933.33 ± 9199.66	
Intercept ± SD	: 42115.67 ± 818.61	
	: 0.9997	
(LOD)	: 0.00648	
(LOQ)	: 0.01965	
(Range)	: 0.02-250	

**Mean of three replicates*

Table 3: Precision and Accuracy Studies of Pterostilbene.						
Concentration (µg/ml)	**Intra-day Precision**			**Inter-day Precision**		
	*** Measured Concentration (µg/ml) ± SD**	**%RSD**	**SEM**	*** Measured Concentration (µg/ml) ± SD**	**%RSD**	**SEM**
10	9.99 ± 0.03	0.03	0.0015	9.75 ± 0.04	0.02	0.0012
20	19.97 ± 0.02	0.02	0.0021	19.98 ± 0.13	0.67	0.0768
50	50.07 ± 0.07	0.15	0.0424	49.72 ± 0.26	0.53	0.1513
Accuracy						
Spiked Concentration (µg/ml)	**Total Concentration (µg/ml)**	*** Concentration Found (µg/ml) ± SD**		**%RSD**	**SEM**	**%Recovery**
16 (80 %)	36	35.61 ± 0.0568		0.16	0.0911	98.91
20 (100 %)	40	39.64 ± 0.0210		0.05	0.0303	99.11
24 (120 %)	44	43.82 ± 0.2109		0.48	0.2767	99.59

**Mean of three replicates*

The robustness of the method was estimated by assaying the sample in diverse analytical conditions by deliberately making slight fluctuations the original condition. From the results (Table 4), it was shown that the system suitability parameters, retention times and the assays for the test solution was not much affected there by signifying that the method is robust.

Stress degradation studies

The stability-indicating capability of the method was established from the separation of PTB peak from the degradation peaks of degraded samples. Figure 5 has shown the evidence of chromatograms of the stressed samples. PTB shows sufficient degradation in acidic and alkaline stress conditions, which resulted in the formation of degradant peaks at 3.207, 4.288 and 4.578 min in acid degradation and at 4.602 and 5.492 min during alkaline degradation. The drug peak was separated properly with better resolution from the so formed degradants, for which the system suitability parameters were within the acceptance criteria as shown in Table 5. The 3D chromatograms for the degradation studies were obtained from the PDA data which shows the selectivity of the wavelength and the degradation peaks at the wide range of wavelength (Figure 4a-4g). During the optimization of the conditions for oxidative stress

Table 4: Robustness Study of Pterostilbene.

Parameter (condition)	*%Assay ± SD	%RSD	SEM	*Retention time ± SD	%RSD	SEM
Mobile phase flow rate (± 10%)						
(0.54 ml/min)	100.00 ± 0.0121	0.01	0.0070	5.19 ± 0.0154	0.2965	0.0089
(0.66 ml/min)	99.02 ± 1.6933	1.71	0.9776	4.89 ± 0.0172	0.3521	0.0099
Detection wavelength (± 2 nm)						
(217 nm)	98.03 ± 1.6902	1.72	0.9758	5.21 ± 0.0065	0.1248	0.0038
(221 nm)	99.85 ± 0.0364	0.04	0.0210	5.20 ± 0.0036	0.0693	0.0021
Mobile phase composition (0.1% TFA: Acetonitrile) (±2, v/v)						
(12:88, v/v)	99.99 ± 0.0099	0.01	0.0057	5.22 ± 0.0316	0.6042	0.0182
(08:92, v/v)	99.81 ± 0.0634	0.06	0.0366	5.20 ± 0.0032	0.0619	0.0019

**Mean of three replicates*

Table 5: Forced Degradation Studies of Pterostilbene.

Stress Conditions	Retention time (R_t)	*Drug recovered (%)	*Drug decomposed (%)	Theoretical plates (N)	Tailing factor	Extra peaks	Resolution (R)
Standard	5.178	100.00	0.00	15342.694	1.269	-	-
Acidic degradation	5.154	92.35	7.65	15453.164	1.278	3.207	3.497
						4.228	
						4.578	
Alkaline degradation	5.171	95.43	4.57	14508.194	1.273	4.602	2.797
						5.492	
Oxidative degradation	5.175	93.01	6.99	15602.304	1.274	-	-
Thermal degradation	5.203	97.21	2.79	14911.933	1.279	-	-
Hydrolysis	5.137	99.13	0.87	14323.968	1.261	-	-
Photolytic degradation	5.123	96.37	3.63	14912.366	1.266	-	-

**Mean of three replicates*

Table 6: Assay of Available Formulations.

Formulation	Labelled claim (mg)	Amount found* (mg)	% Recovery*
I	50	49.38	98.75
II	50	49.37	98.94

degradation study, the strength of the Hydrogen peroxide was varied for 3% Hydrogen peroxide and finally to 30% Hydrogen peroxide. Even at the final condition the drug have shown almost no degradation. As mentioned in the ICH Q1B Guidelines,[20] for photo-stability forced degradation studies, a variety of exposure conditions may be used, depending on the photo sensitivity of the drug. It is appropriate to limit the exposure and end the study if extensive degradation occurs. But in our case, the dug has almost shown no degradation even exposed for 7 days.

Analysis of commercial formulations

The proposed method was applied to the assay of available formulations for the determination of PTB. The % recovery was found to be 98.75-98.94 (Table 6). The resultant chromatograms obtained for the assay of marketed formulations were shown in Figure 3c-3d.

CONCLUSION

The proposed stability-indicating HPLC method for the determination of PTB in pharmaceutical dosage forms was developed and validated as per ICH guidelines. The water used in this method development is of HPLC grade and was procured from Merck (India). The method has produced precise results during the inter-day precision studies which was conducted in 3 different days, which implies that the water quality shows no effect in the method development.. From the forced degradation studies it was clear that PTB was tends to be more sensitive towards acidic and alkaline degradations. The drug peak was sufficiently resoluted for the

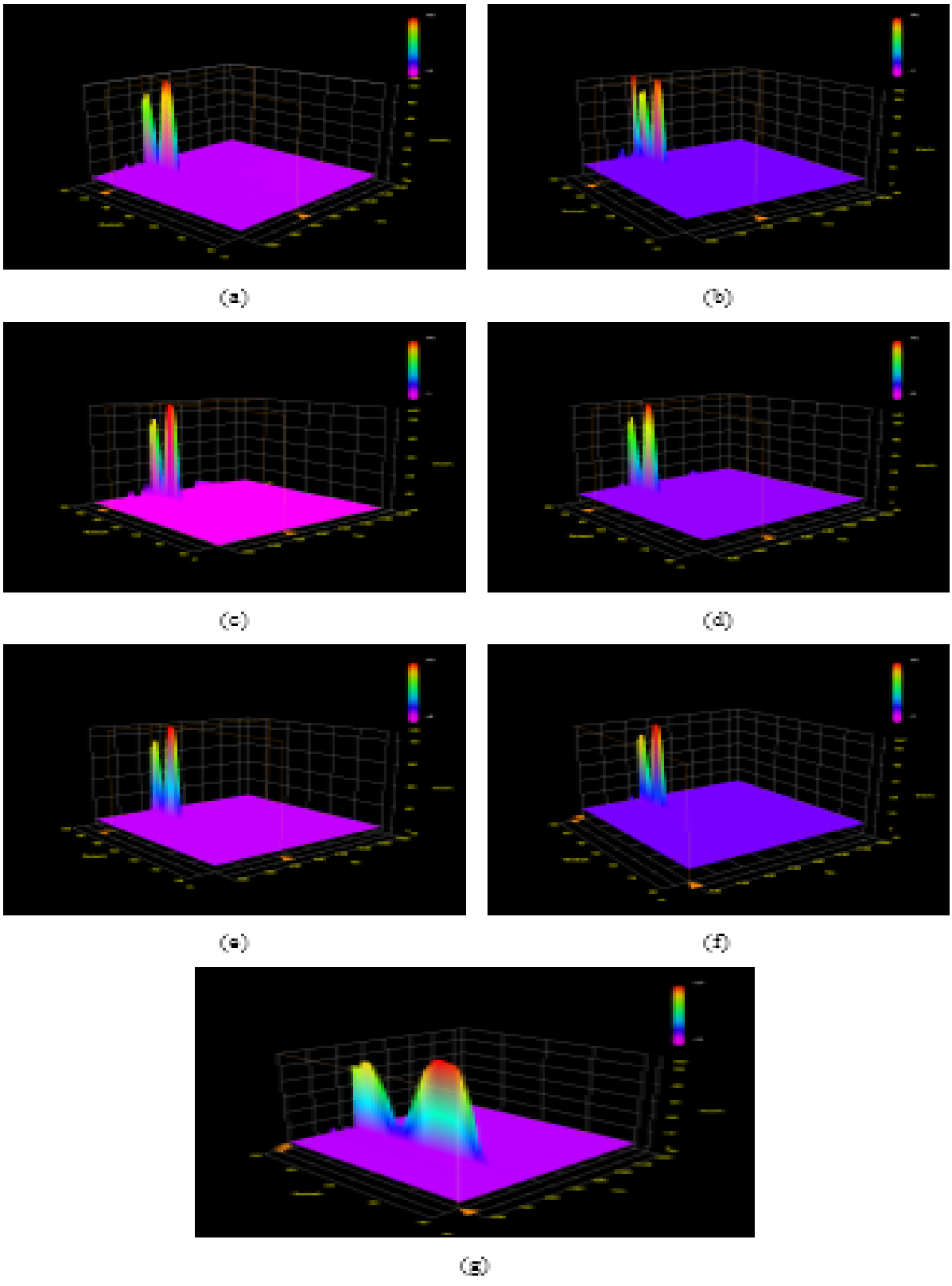

Figure 4: 3D Chromatograms of Pterostilbene (a) Standard (20 µg/ml) (b) Alkaline (c) Acidic (d) Oxidative (e) Photolytic (f) Thermal and (g) Hydrolysis degradations

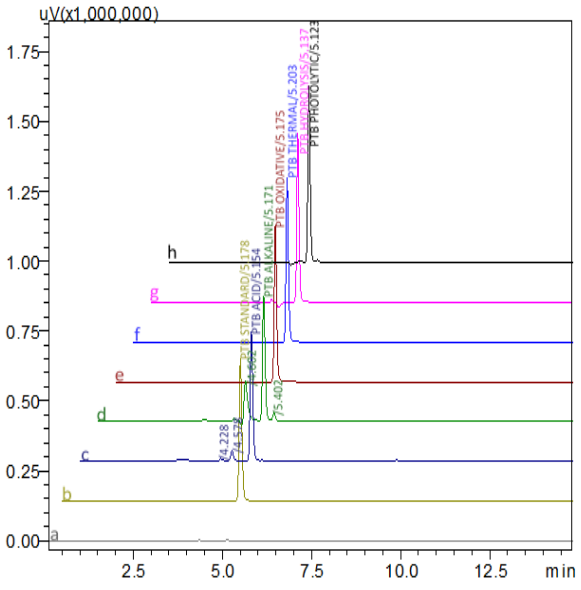

Figure 5: Typical chromatograms of Pterostilbene (a) Blank (b) Standard (20 µg/ml) (c) Acidic (d) Alkaline (e) Oxidative (f) Thermal (g) Hydrolysis (h) Photolytic degradations

so formed degradants obtained during stress degradation studies. The developed method was successfully applied to the available formulations. This method can be helpful for the long term stability studies and also to the kinetics studies.

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CONFLICT OF INTEREST

Authors decalare no conflict of interest.

ABBREVIATIONS

PTB: Pterostilbene; **RP:HPLC:** Reverse Phase High Performance Liquid Chromatography; **TFA:** Trifluoroacetic acid; **ACN:** Acetonitrile; **NaOH:** Sodium hydroxide; **HCl:** Hydrochloric acid; **H2O2:** Hydrogen peroxide; **ICH:** International Conference on Harmonization; **RSD:** Relative Standard Deviation; **LOQ:** Limit of Quantitation; **LOD:** Limit of Detection

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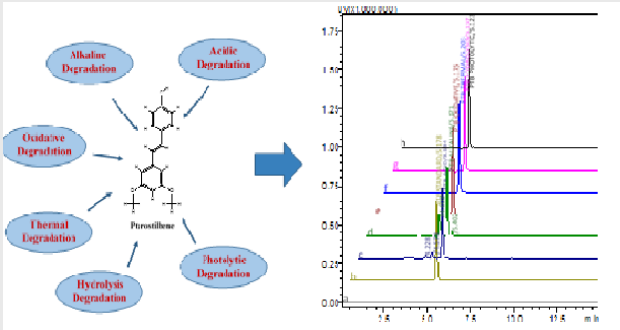
PICTORIAL ABSTRACT



SUMMARY

- Pterostilbene is a stilbenoid, with diversified pharmacological activities such as anti-oxidant, anti-inflammatory, anti-diabetic, anti-cancer etc.
- A new stability-indicating RP-HPLC method has been developed and validated (ICH guidelines) for the determination of Pterostilbene.
- Optimized chromatographic conditions –
- Mobile Phase: Trifluoroacetic acid in water: Acetonitrile (10:90 %, v/v)
- Flow rate: 0.6 ml/min (UV detection at 219 nm).

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