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A New Absorption Subtraction Method And Validation Of Simultaneously Estimation Of Resveratrol And Benzoyl Peroxide By Uv Spectrophotometric Method

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ABSTRACT : A new simple, sensitive, spectrophotometric method in UV region has been developed for the simultaneous estimation of benzoyl peroxide and resveratrol. The method involved absorption subtraction method using two wavelengths, with one being of benzoyl peroxide (234 nm,) and the other being the isoabsorptive point of both drugs (246 nm). The Standard solution of benzoyl peroxide and resveratrol shows maximum absorbance at 234 nm and 306 nm respectively. Beer's Lamberts law is obeyed in concentration range 1-7 µg/ml for resveratrol while for benzoyl peroxide, Beer's Lambert law is obeyed in concentration range 1-10 µg/ml. The results of analysis have been validated statistically and by recovery study. The accuracy ranged for resveratrol was between 103.16 and 104.49% and for benzoyl peroxide 104.05 to 110.49. The method was found to be precise, reproducible and rapid.

Keywords: Benzoyl peroxide, Resveratrol, Simultaneous estimation , absorption subtraction method.

1.INTRODUCTION

Chemically, Resveratrol(RES) is 3,5,4'-trihydroxy-trans-stilbene,a type of natural phenol and a phytoalexin produced naturally by several plants in response to injury or when the plant is under attack by pathogens such as bacteria or fungi. It is a found in red wine, colour berries, and inedible parts of the peanut plant[1]

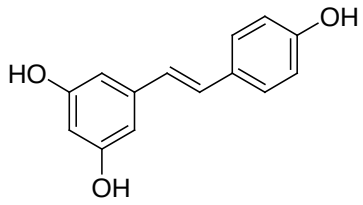


Figure 1: Chemical Structure of Resveratrol

It is a powerful antioxidant produced by some plants to protect them against environmental stresses .Resveratrol has been shown to have a variety of therapeutic properties including

antioxidant, anti-inflammatory, antimicrobial, antineoplastic, and wound healing activity.[2] Studies have found RES to be one of the strongest antioxidants, stronger than Vitamin A, C, and E [3]. Antioxidants reduce lipid peroxidation and the development of reactive oxygen species, which cause tissue inflammation and DNA damage. Resveratrol is also known to have antiviral, antifungal, antibacterial, and antiprotozoal effects.[4-5]

Benzoyl peroxide (BPO) is an organic compound in the peroxide family. It consists of two benzoyl groups bridged by a peroxide link. Its structural formula is $[C_6H_5(CO)_2]O_2$. It is one of the most important organic peroxides in terms of applications and the scale of its production. Benzoyl peroxide is a potent antibacterial agent which is the first line drug in treatment of acne vulgaris [6]. The chemical structure of Resveratrol and Benzoyl peroxide is shown in figure 1 and figure2.

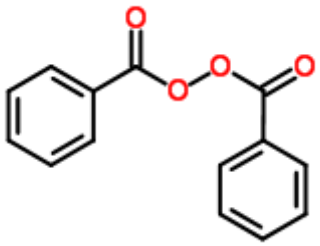


Figure 2: Structure of benzoyl peroxide

The combination of both drugs would be beneficial for the treatment of mild to moderate stage of acne vulgaris.[7]. However for the combination therapy of two drugs if they are administered in the form of a single formulation, a simultaneous estimation of the two would be required. Literature survey revealed that there was no validated UV method for the estimation of resveratrol and benzoyl peroxide simultaneously by UV spectrophotometric method .The present study is aimed at to develop selective, precise , accurate and reliable UV method for determination of Benzoyl peroxide and resveratrol in a mixture .

2. MATERIAL AND METHOD

2.1 Reagents and Apparatus: Benzoyl peroxide and Resveratrol were obtained as gift samples from JIGS chemicals and avanscure life sciences pvt .Ltd respectively. All other chemicals used were of analytical grade. A double beam UV-Visible spectrophotometer, model 1800, Shimadzu, Japan with 1 cm quartz cell was used for all analysis.

3.EXPERIMENTAL

3.1) PREPARATION OF STANDARD STOCK SOLUTIONS

Standard stock solution (100μg/ml) of Benzoyl peroxide and Resveratrol was prepared separately by dissolving carefully weighed 10mg of drug in 100ml volumetric flask and diluting up to the mark with methanol. 1 ml of this solution was diluted up to 10ml with methanol to get working stock solution (10μg/mL).

2) DETERMINATION OF ISOABSORPTIVE POINT AND WAVELENGTH OF MAXIMUM ABSORBANCE (λMAX).

Solutions of 10 μg/ml of both drugs were prepared from working stock solution and scanned in the range of 200nm to 400nm against methanol as blank. The overlaying spectrum was also obtained to determine isoabsorptive point.(Fig 3)

3) STANDARD CALIBRATION CURVE (LINEARITY)

A calibration curve was plotted over a concentration range of 1–10 μg/ml for Benzoyl peroxide, 1-7 μg/ml for resveratrol and 1-10 μg/ml for mixture of benzoyl peroxide and resveratrol (1:1) . Accurately measured stock solution of Benzoyl peroxide (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0ml), stock solution of resveratrol (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 ml) and stock solution of Benzoyl peroxide and resveratrol (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0ml) were transferred to separate series of 10ml volumetric flask and diluted up to the mark with methanol. The absorbance of all solutions was taken at their respective λmax and at isoabsorptive point. The calibration curves were constructed by plotting concentration against absorbance where each reading was an average of three determinations.

ABSORPTION SUBTRACTION METHOD is used [8,9]. This method is based on the same principles as the absorption factor method and it depends on that, if there is a mixture of two drugs X and Y having overlapped spectra intersect at isoabsorptive point and Y is extended more than X, while X doesn't show any absorbance (A_2) at another wavelength (λ_2).

In this method the isoabsorptive point λiso could be used for separate quantitative estimation of each X & Y in their mixture (X+Y). The determination was done by using mathematically

calculated factor of one of these components. By simple manipulation step, the absorbance values corresponding to X and Y, separately could be obtained. So, the concentration of each component could be obtained via the isoabsorptive point regression equation without any need for a complementary method.

The absorbance values corresponding to X and Y at λiso were calculated by using absorbance factor (Aiso /A2) which is a constant for pure Y representing the average of the ratio between the absorbance values of different concentrations of pure Y at λiso (Aiso) to those at λ_2(A2) .

Absorbance of Y in the mixture at λiso = abs1/ abs2 × abs λ_2(X+Y)

Absorbance of X in the mixture at λiso = abs λiso (X+Y) − abs1/ abs2× abs λ_2 X+Y

Where; abs1, abs2 is the absorbance of pure Y at λiso and λ_2 ; abs1/ abs2 is called the absorbance factor and abs λiso (X + Y) and abs λ_2(X+ Y) are the absorbance of the mixture at these wavelengths (λiso, λ_2).

The concentration of each X or Y, separately, is calculated using the isoabsorptive point unified regression equation {obtained by plotting the absorbance values of the zero order curves of either X or Y at isoabsorptive point (λiso) against their corresponding concentrations X or Y respectively}.

4. ANALYTICAL VALIDATION BY UV SPECTROSCOPIC METHOD

4.1 LINEARITY AND RANGE

Linearity, consisting of the basic elements input → converter → output, is the assumption that there is a straight line relationship between the input and output variables that can be written mathematically by the expression if the straight line crosses through the origin or by the expression if the straight line does not cross through the origin. The linear range corresponds to the valid interval of functional dependence of the signal on concentration or mass which assumes homoscedasticity of the measurements over the linear range. The linear response of BPO and RES was determined by analyzing the calibration curve in the range of 1-10 µg/mL and1-7µg/ml.

4.2 PRECISION.

The term precision is defined by the ISO International Vocabulary of Basic and General Terms in Metrology (ISO-VIM) and ICH as the closeness of agreement between quantity values obtained by replicate measurements of a quantity under specified conditions [10]. Assessing the precision implies expressing numerically the random error or the degree of dispersion of a set of individual measurements by means of the standard deviation, the variance, or the coefficient of variation.

4.3 Repeatability (Within-Run Precision). It is the concordance of a series of measurements of the same quantity when the experiments are conducted under same conditions (analyst, apparatus, instrument, and day) in a rapid succession. For this experiment, standard solution of Benzoyl peroxide and Resveratrol (3 + 3 μg/mL) was prepared and analyzed six times as per the proposed method.

4.4 Intermediate Precision (Between-Run Precision). It is the concordance of a series of measurements of the same quantity when the experiments are conducted within the same laboratory under different conditions (analyst, apparatus, instrument, and day). Standard solution of Resveratrol and Benzoyl peroxide (3 + 3 μg/mL) was prepared and analyzed as per the proposed method.

4.4 Accuracy (%recovery)

The accuracy was tested by recovery experiments. Recovery studies were carried out at 100 % level by adding a known quantity of pure drug to the preanalyzed formulation and the proposed method was followed. From the amount of drug found, percentage recovery was calculated.

Process: The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The recovery experiments were carried out in triplicate by spiking previously analyzed samples with three different concentrations of standards.

4.5 Ruggedness

Ruggedness was determined by carrying out analysis by two different analyst and the respective percentage recovery was noted and the results was indicated as % RSD .

4.6 Limit of Detection (LOD) and Limit of Quantification (LOQ).

The detection limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in the sample which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ of the proposed method were determined by using calibration curve:

$$\text{LOD} = \frac{3.3\sigma}{S}, \qquad \text{LOQ} = \frac{10\sigma}{S},$$

Where σ is the standard deviation of the response (Y intercept) and S is the slope of the calibration curve.

4.5 Specificity

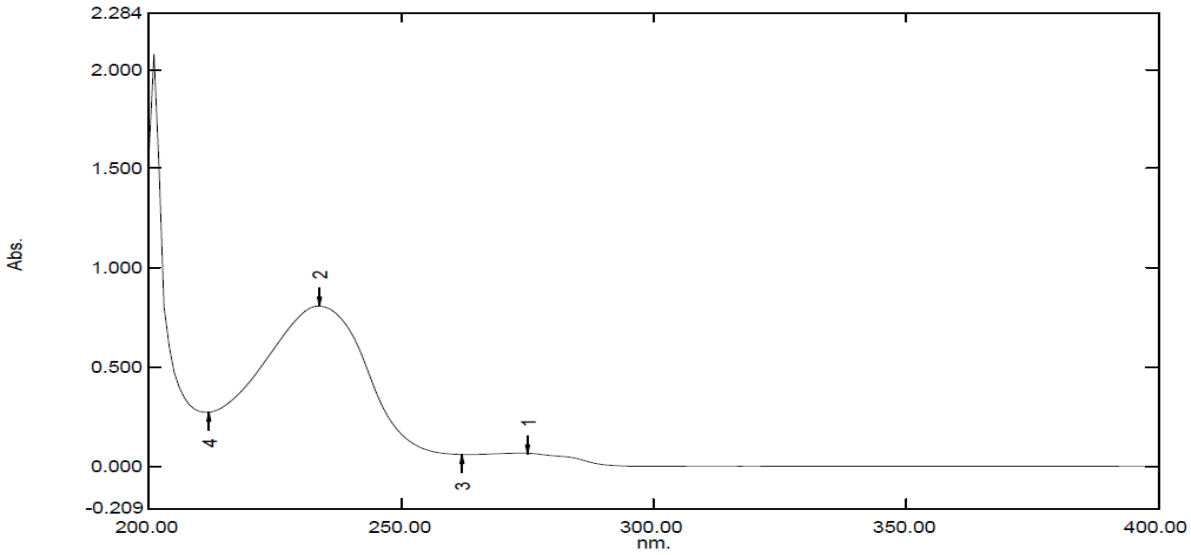
Specificity of the methods was achieved by the analysis of different laboratory prepared mixtures of resveratrol and benzoyl peroxide within the linearity range.

5. RESULTS AND DISCUSSION

The solutions of 10 μg/mL of both benzoyl peroxide and resveratrol were analyzed and the λ_{max} was found to be 234 nm and 306 nm, respectively. Two isoabsorptive points: 246nm and 230 nm were found in overlaying spectra (Figure 3,4) .But 246nm was selected for further analysis. The calibration curve of BPO and RES individually and the mixture of both drugs at 234 nm (Fig 4) and 306 nm (fig) were plotted (Figures 4,5 and 6). The relationship between the absorbance and the concentration of benzoyl peroxide and Resveratrol was found to be linear in the range of 1-10 g/mL at wavelengths 234 nm and in the range of 1-7mcg/ml at wavelength 306 nm respectively. The representative linear equations and correlation coefficients have indicated very good linearity (Table 1). Evaluation of repeatability and intermediate precision was done and coefficients of variation (CV) or percent relative standard deviation (%RSD) values were calculated. These values were found to be less than two (CV < 2), indicating good precision (Table 2). Good accuracy of the proposed method was proved by good percent recovery in standard addition method. It ranged between 103.16 and 104.49% for benzoyl peroxide and 104.05 and 110.49% for resveratrol (Table 3).

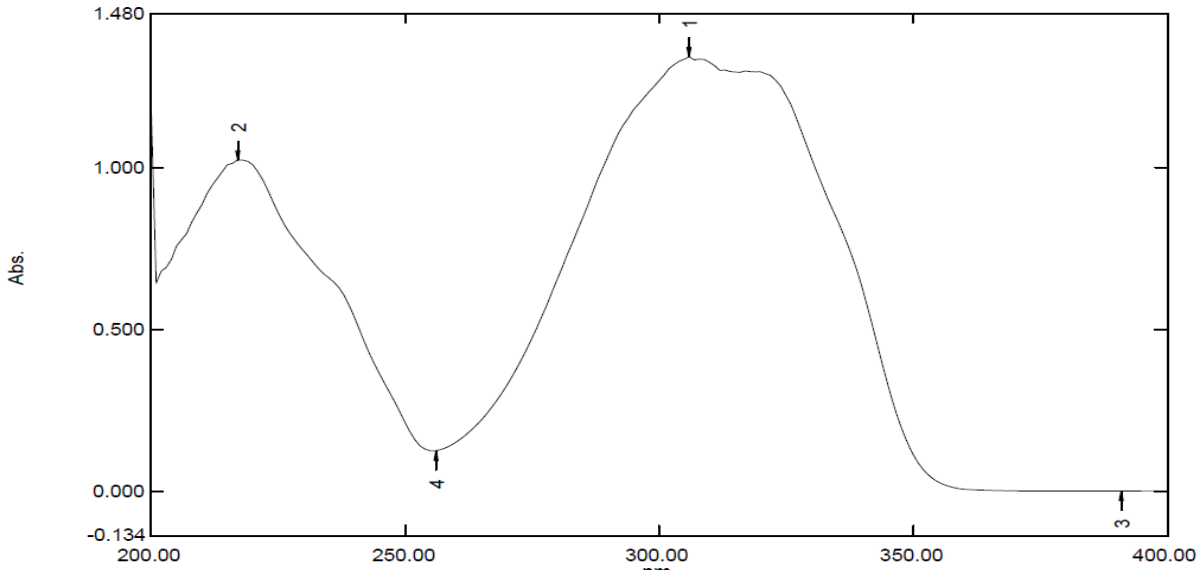
The limit of detection of BPO and RES at isoabsorptive point (246 nm) was found to be 0.133 µg/mL and 0.080 µg/mL. The LOD of benzoyl peroxide at 234 nm was found to be 0.109 µg/mL and of resveratrol at 306 is 0.029 µg/mL . The limit of quantification of BPO

and RES at isoabsorptive point (246 nm) was found to be 0.404 µg/mL and 0.243 µg/mL. The LOQ of benzoyl peroxide at 234 nm was found to be 0.331 µg/mL and of resveratrol at 306 0.089 µg/mL ..



No.	P/V	Wavelength	Abs.	Description
1	↑	275.00	0.066	
2	↑	234.00	0.807	
3	↓	262.00	0.059	
4	↓	212.00	0.271	

FIGURE 1: ABSORPTION MAXIMA OF BENZOYL PEROXIDE PURE DRUG



No.	P/V	Wavelength	Abs.	Description
1	↑	306.00	1.345	
2	↑	217.00	1.026	
3	↓	391.00	0.000	
4	↓	256.00	0.126	

FIGURE 2 : ABSORPTION MAXIMA OF RESVERATROL PURE DRUG

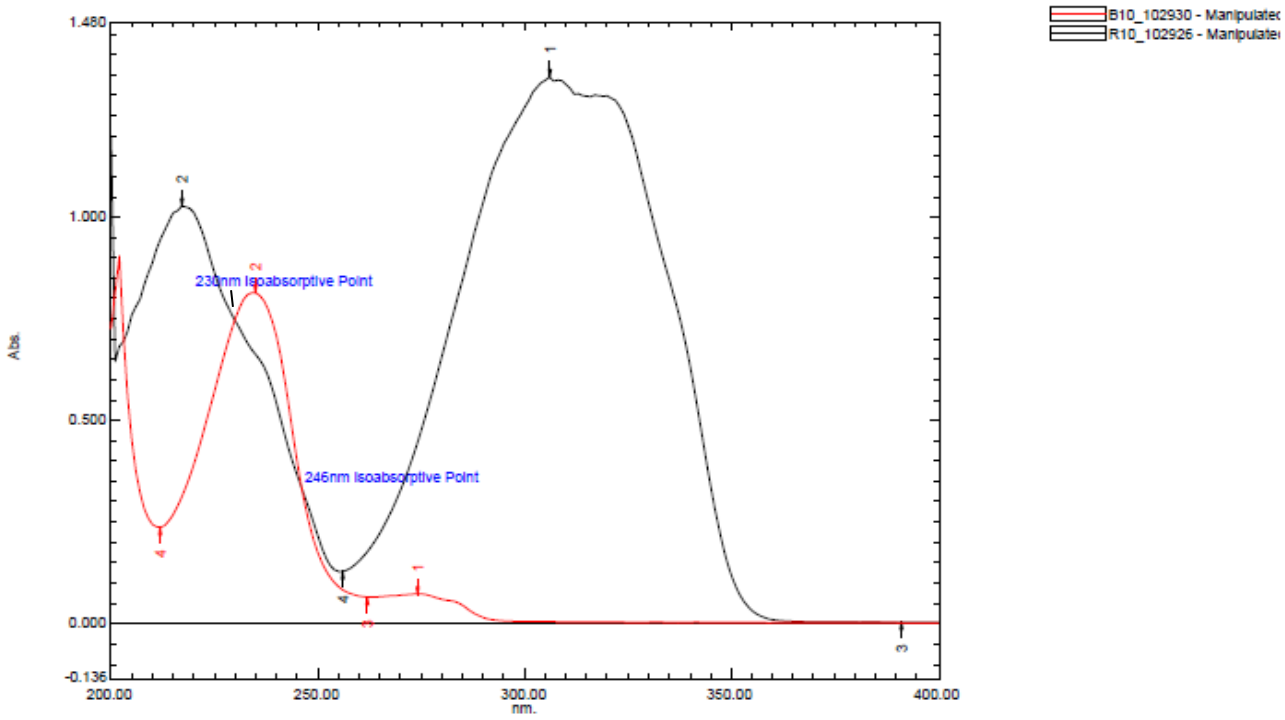


FIGURE 3: OVERLAY GRAPH OF BENZOYL PEROXIDE AND RESVERATROL SHOWING ISOABSORPTIVE POINTS.

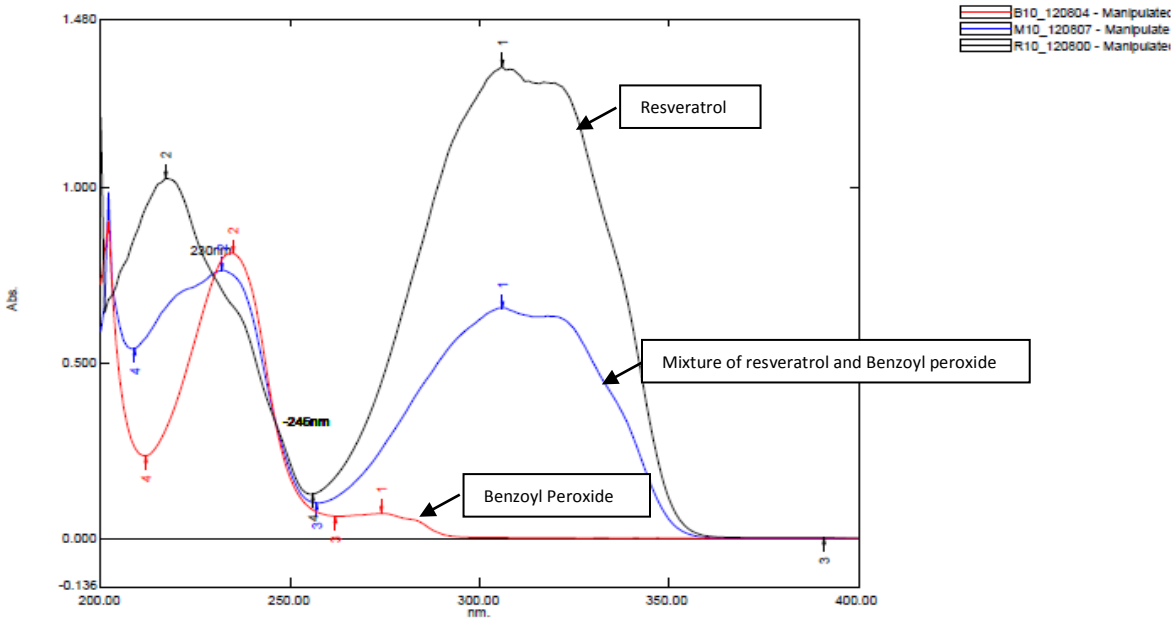


FIGURE 4 : OVERLAY GRAPH OF BENZOYL PEROXIDE AND RESVERATROL AND MIXTURE SHOWING ISOABSORPTIVE POINTS.

Table 1: **Standard calibration curve of Benzoyl peroxide at 234nm**

S.no.	Con.(µg/ml)	Absorbance at 234nm ±STD	% RSD
1	1	0.11167±0.0020	1.864179
2	2	0.1813±0.0020	1.147978
3	3	0.27167±0.0040	1.487651
4	4	0.35133±0.0030	0.869559
5	5	0.419±0.0034	0.826755
6	6	0.50233±0.0028	0.574668
7	7	0.56267±0.00208	0.369964
8	8	0.638±0.001	0.15674
9	9	0.72467±0.0066	0.918813
10	10	0.805±0.002	0.248447

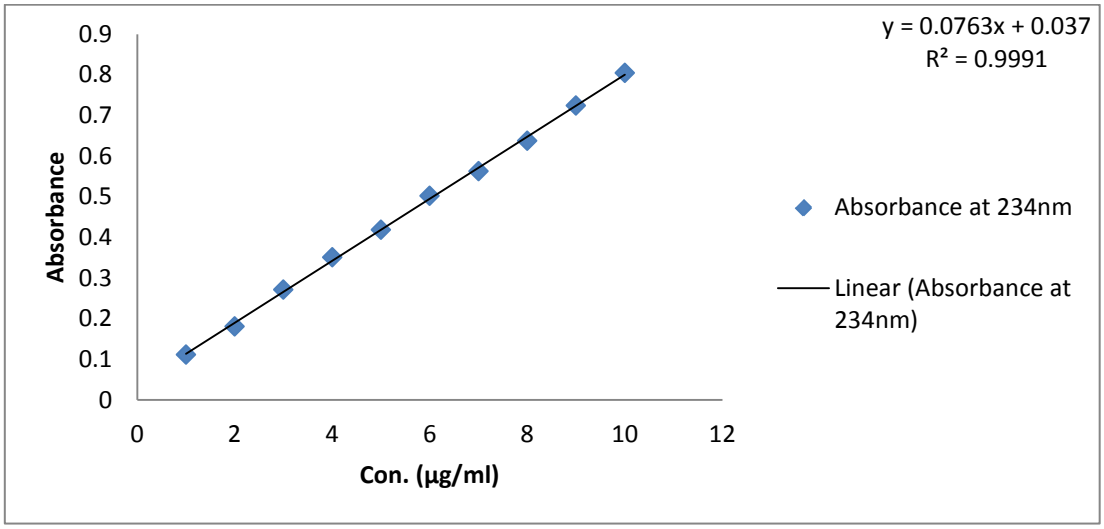


Figure 5: Standard calibration curve of Benzoyl peroxide at 234nm

Table 2 : Standard calibration curve of Benzoyl peroxide at Isoabsorptive point 246nm

S.no.	Conc.(µg/ml)	Absorbance at 246nm (Isoabsorptive point)STD	% RSD
1	1	0.04733±0.0005	1.219754
2	2	0.078±0.0026	3.391989
3	3	0.10967±0.0030	2.78576
4	4	0.14067±0.0025	1.78906
5	5	0.16933±0.0028	1.704774
6	6	0.20467±0.0023	1.128372
7	7	0.22633±0.0005	0.255088
8	8	0.25866±0.0005	0.223202
9	9	0.293±0.0034	1.182287
10	10	0.31867±0.0023	0.724707

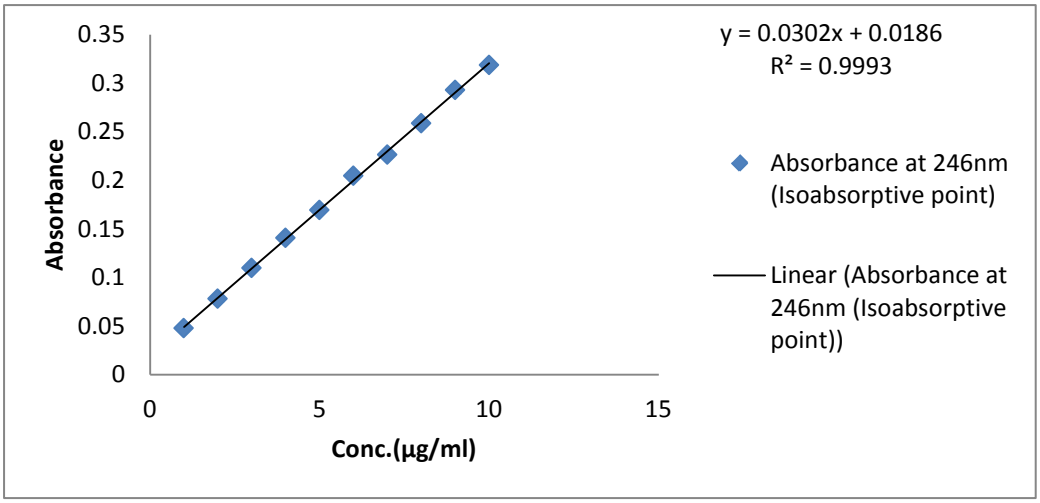


Figure 6: Standard calibration curve of Benzoyl peroxide at 246nm

5) Table 3: **Standard calibration curve of Resveratrol at 306nm**

S.no.	Con.(µg/ml)	Absorbance at 306nm	RSD
1	1	0.13733±0.0011	0.840801
2	2	0.27033±0.0011	0.42714
3	3	0.40733±0.0020	0.511047
4	4	0.52966±0.0025	0.475131
5	5	0.66466±0.0005	0.086863
6	6	0.79566±0.00251	0.31629
7	7	0.91066±0.00230	0.253595

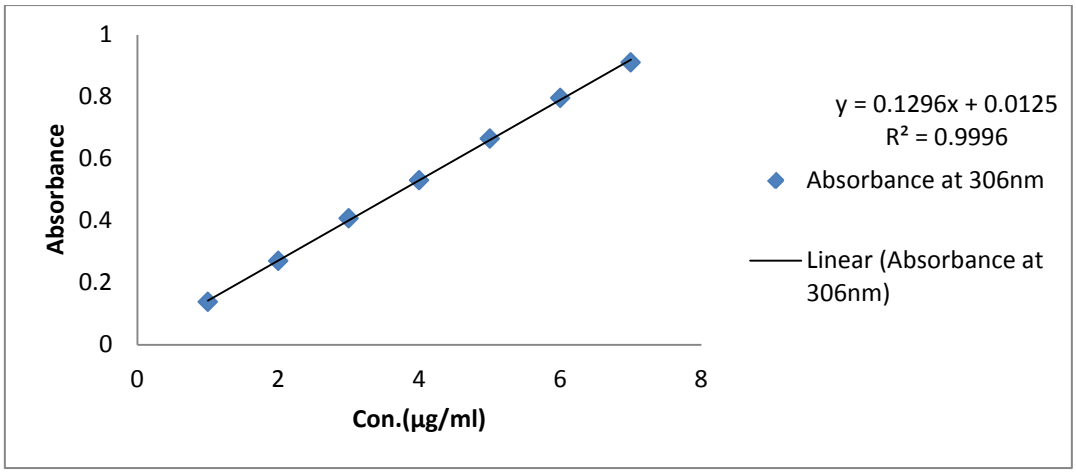


Figure 7: Standard calibration curve of resveratrol at 306nm

6) Standard calibration curve of Resveratrol at 246 nm

Table 4: **Standard calibration curve of Resveratrol at 246 nm**

1	1	0.036±0.0005	1.57459
2	2	0.068666667±0.00152	2.224551
3	3	0.104666667±0.00230	2.206434
4	4	0.138±0.00173	1.255109
5	5	0.167666667±0.00057	0.344344
6	6	0.197±0.00173	0.879214
7	7	0.224333333±0.00057	0.256981

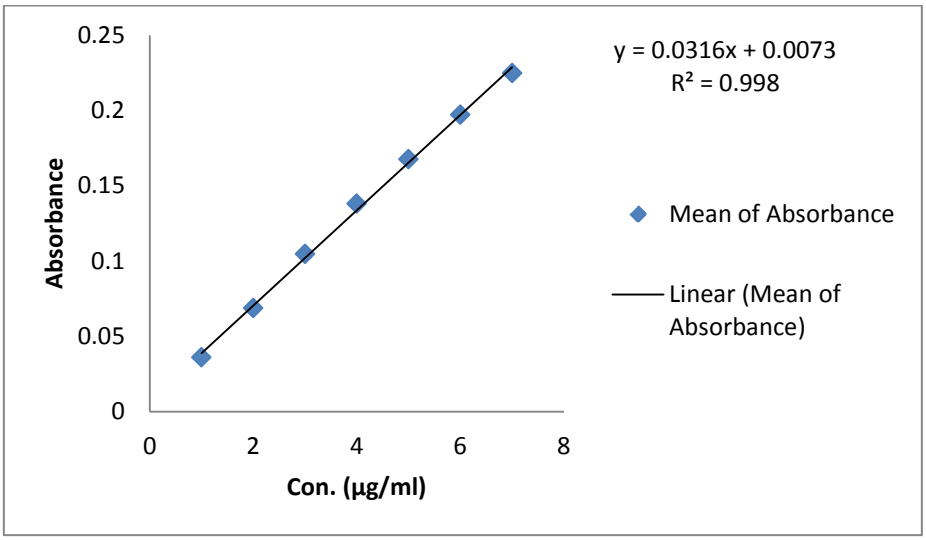


Figure 8: Standard calibration curve of resveratrol at 246nm

Precision

Table 5 : Repeatability and Intermediate Precision study

S.no.	Precision	Percentage recovery of Resveratrol	% RSD	Percentage recovery of benzoyl peroxide	% RSD
1	Repeatability	105.027± 0.748	0.712	100.762±1.280	1.270
2	Intermediate Precision (Day1-Day 6)	104.984±1.560	1.486	101.979±0.161	0.157

Accuracy

Table 6: Results of recovery studies of resveratrol and Benzoyl peroxide.

S.N	Amount of mixture taken	Amount of Resveratrol added	Percentage recovery of resveratrol	% RSD
1	3+3mcg/ml	1 (µg/ml)	103.166±0.708	0.686
2	3+3mcg/ml	2 (µg/ml)	103.753±0.272	0.262
3	3+3mcg/ml	3 (µg/ml)	104.493±0.619	0.592

S.N.	Amount taken of mixture	Amount added of Benzoyl peroxide	Percentage recovery of Benzoyl peroxide	% RSD
1	3+3mcg/ml	1 (µg/ml)	110.498 ± 2.117	1.915
2	3+3mcg/ml	2 (µg/ml)	106.587 ± 1.385	1.299
3	3+3mcg/ml	3 (µg/ml)	104.054 ± 0.431	0.414

Ruggedness

Ruggedness was determined by carrying out analysis by two different analyst and the respective percentage recovery was noted and the results was indicated as % RSD .

Table 7: Results of Ruggedness of resveratrol and Benzoyl peroxide.

S.no.		Percentage recovery of Resveratrol	% RSD	Percentage recovery of benzoyl peroxide	% RSD
1	Analyst 1	105.027± 0.748	0.712	100.762± 1.280	1.270
2	Analyst 2	107.036± 0.657	0.614	102.981±2.050	1.991

Table 8: Limit of Detection (LOD) and Limit of Quantification(LOQ).

s.no.	Name of drug	234nm		306nm		246nm	
		LOD (µg/ml)	LOQ(µg/ml)	LOD (µg/ml)	LOQ(µg/ml)	LOD (µg/ml)	LOQ(µg/ml)
1	Resveratrol	-		0.029	0.089	0.080	0.243
2	Benzoyl peroxide	0.109	0.331	-	-	0.133	0.404

Specificity

Specificity of the methods was achieved by the analysis of different laboratory prepared mixtures of resveratrol and benzoyl peroxide within the linearity range.

Table 9 : Specificity of different laboratory prepared mixture

S.no.	Ratio	Percentage recovery of Resveratrol	% RSD	Percentage recovery of benzoyl peroxide	% RSD
1	Resveratrol : Benzoyl peroxide (2:1)	104.231± 0.646	0.619	97.014± 2.306	2.377
2	Resveratrol : Benzoyl peroxide (1:2)	103.9362± 0.302	0.291	110.864± 0.503	0.454

.5. CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

6. CONCLUSION

The UV spectrophotometric absorption subtraction method was developed and validated for the simultaneous analysis of Benzoyl peroxide and Resveratrol. The results together established that the method is simple, accurate, precise, reproducible, rapid, and sensitive. The method could be applied successfully and economically for the simultaneous estimation of BPO and RES in laboratory samples for efficient data generation and for combination formulations of these two drugs in the future.

7.REFERENCES :

[1] Baur JA, Sinclair DA. Therapeutic potential of resveratrol: the in vivo evidence. Nat Rev Drug Discov 2006 ; 5: 493-506.

[2] BaxterRA. Anti-aging properties of resveratrol: review and report of a potent new antioxidant skin care formulation. J Cosmet Dermatol 2008;7: 2-7.

[3] Docherty JJ, Smith JS, Fu MM, StonerT, Booth T. Effect of topically applied resveratrol on cutaneous herpes simplex virus infections in hairless mice.2004; Antiviral Res 61: 19-26 .

[4] ChanMM. Antimicrobial effect of resveratrol on dermatophytes and bacterial pathogens of the skin.2002; Biochem Pharmacol . 63: 99-104 .

[5] Kedzierski L,Curtis JM, Kaminska M, Jodynis-Liebert J, Murias M. In vitro antileishmanial activity of resveratrol and its hydroxylated analogues against Leishmania major promastigotes and amastigotes.2007; Parasitol Res. 102: 91-97.

[6] Gollnick H, Cunliff W, Berson D, et al.2003; J. Am. Acad. Dermatol.49,1: S1-S37.

[7] Leyden JJ.2003; J.Am.Acad. Dermatol. 49(3): S200-S210.

[8] KhadigaM, Abdallah A, MagdaY, Michael K. Spectrophotometric and Chemometric Methods for Simultaneous Determination of Two Anti-Hypertensive Drugs in their Combined Dosage Form .2015 ;Pharm Anal Acta . 6:1.

[9] Lotfy HM, Amer SM, Zaazaa HE and Mostafa NS, Spectrophotometric Methods for Quantitative determination of Binary Mixture of Naproxen Sodium and Domperidone Maleate.2015; Austin J Anal Pharm Chem - 2(3):13.

[10]A. Krautheim and H.P. Gollnick, Clindermatol. 22, 398-407 (2004).