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## SPECTROSCOPIC METHODS FOR DETERMINATION OF BUTYLATED HYDROXYANISOLE (BHA)

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### ABSTRACT

analytical methods viz. Spectrophotometric methods (A and B) which being employed for analysis, are relatively expensive and hence need for simple analytical methods arises. In the proposed research work, such methods have been developed and applied for routine determination of Butylated hydroxy anisole (BHA) in pharmaceutical formulations and bulk dosage forms. These methods were based on the formation of colored species on binding of ferrous ions with 1,10- Phenanthroline in the presence of Orthophosphoric acid for method A and formation of a colored chromogen on binding of ferrous ions with, 2,2-bipyridine in the presence of Orthophosphoric acid for method B, and the colored chromogen obtained in each method was finally treated with the antioxidant BHA to produce orange red color for method A and orange colored chromogen for method B with  $\lambda_{max}$  at 510 nm and 520 nm respectively. Statistical analysis of these methods exhibited Sandal's Sensitivity of 0.026 and 0.038 (Method A and B) respectively, and the relative standard deviation (RSD) of these methods were found equal to 1.60 and 1.87 respectively for method A and method B, indicating that these methods are reproducible, based on the principle of absorption visible spectrophotometry for the determination of BHA in formulations and bulk dosage forms.

**Keywords:** BHA, Spectrophotometer, Molar Absorptivity, Beer's Law, Assay, Recovery

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### INTRODUCTION

#### Butylated Hydroxy Anisole (BHA):

BHA ( $\text{CH}_3\text{C}_6\text{H}_3(\text{OH})\text{C}(\text{CH}_3)_3$ ) is an antioxidant consisting of a mixture of two isomeric organic compounds, 2-tert-butyl-4-hydroxyanisole and 3-tert-butyl-4-

hydroxyanisole (Retrieved Nov 2009))<sup>[1]</sup>. BHA can induce allergic reactions in the skin (U.S. National Library of Medicine, (2010))<sup>[2]</sup>. It is a lipophilic (fat-soluble) organic compound that is primarily used as an antioxidant food additive

as well as an antioxidant additives in cosmetics, pharmaceuticals, jet fuels, rubber, petroleum products, oil and embalming fluid. (Fiche de Datos de Seguridad (2008))<sup>[3]</sup>. BHA has been added to edible fats and fat-containing foods for its antioxidant properties. It is also used in foods cooked or fried in animal oils, because of its high thermal stability and its ability to remain active in baked and fried foods (HSDB (2009))<sup>[4]</sup>.

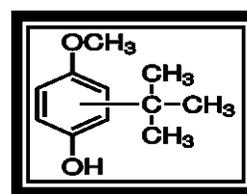
### **Carcinogenicity:**

A population-based nested case-control study of stomach cancer in men and women within the Netherlands Cohort Study of dietary intake found no increase in risk at typical levels of dietary intake of BHA (Botterweck (2000))<sup>[5]</sup>. It can cause or aggravate conditions such as asthma and hives, as well as developmental disorders in children (Lahey, M., Rosen, S., (2007))<sup>[6]</sup>. BHA can block cell respiration by inhibiting the activity of complex I (NADH-CoQ reductase), complex II (succinate-CoQ oxidoreductase) and complex III (cytochrome c-ubiquinole reductase), (Okubo T. (2004))<sup>[7]</sup>.

The reactive oxygen species (ROS) scavenging capacities of BHA are frequently used to argue that ROS have a role in certain signaling pathways. As both compounds decrease the levels of ROS and at the same time protect against TNF-induced necrosis of L929 fibro sarcoma cells, it was concluded that ROS, formed and acting in the hydrophobic environment of the inner mitochondrial

membrane, mediate this cell death process, (Goossens V (1995))<sup>[8]</sup>. The reaction mixture and several products arising from the reaction of BHA and nitrite in anaerobic aqueous acidic solution were separated and tested in the Salmonella mutagenicity test. Among the nine products separable by thin-layer chromatography, 1-hydroxyl-2-tert-butyl-4-methoxy-6-nitrobenzene (BHA-NO<sub>2</sub>), tert-butyl-substituted para-quinone (t-BuQ) and 3-tert-butyl-5-methoxy-1, 2-benzoquinone (t-Bu-o-Q) are dominant. The substances gave no evidence of mutagenicity in the Salmonella typhimurium strains TA 98 and TA 100, either in the standard plate incorporation assay or in the procedure with pre-incubation with or without S<sub>9</sub> mix. In some instances the substances were unstable in the test procedure (Kalus WH (1990))<sup>[9]</sup>.

Dietary exposure to BHA caused benign and malignant tumors of the fore stomach (papilloma and squamous-cell carcinoma) in rats of both sexes and in male mice and hamsters (IARC 1986, Masui (1986))<sup>[10]</sup>. Since 1947, BHA has been added to edible fats and fat-containing foods for its antioxidant properties as it prevents food from becoming rancid and developing objectionable odors (Lam, L. W. Wattenberg (1979))<sup>[11]</sup>.



**FIG 1: STRUCTURE OF BHA**

## MATERIALS AND METHODS

### Instrumentation:

After due calibration of the instrument, spectral and absorbance measurements were made using UV-Visible spectrophotometer model SL-159, Mumbai, India. All the chemicals used were of analytical grade. All the solutions were freshly prepared using millipore double distilled water. Freshly prepared solutions were used for analysis. In the propose methods aqueous solutions of 1,10 phenanthroline (0.2%), Ferric chloride(0.3%w/v) and Orthophosphoric acid(0.5N) were used for method A and 2,2-bipyridine (0.2%), ferric chloride (0.5%w/v) and Orthophosphoric acid (1N) were used for method B.

### Standard and Sample solution of BHA:

About 100mg of BHA was accurately weighed on a digital single pan balance and dissolved in a volumetric flask containing 100ml of methanol to prepare a standard solution with a concentration equal to 1mg/ml and further dilutions are made with the same solvent for this method.

### Preparation of Reagents:

All the chemicals used were of analytical grade. All solutions were freshly prepare with distilled water and always used for analysis. Following aqueous solutions were used:

#### Method-A:

- 1,10-phenanthroline (0.2% W/V)
- FeCl<sub>3</sub> (0.7% W/V)

- Orthophosphoric acid (0.5N)

#### Method-B:

- 2,2-bipyridine (0.01M)
- FeCl<sub>3</sub> (0.005M)
- Orthophosphoric acid (0.1N)

## ASSAY PROCEDURE

### Method A:

Into a series of 25 ml calibrated tubes; FeCl<sub>3</sub> (1ml), 1,10 phenanthroline (1ml) were taken. These tubes were kept in a hot water bath for 10min. at 100°C and then cooled to room temperature. To develop the initial color, Orthophosphoric acid solution(2ml) was added to each tube. Aliquots of 0.4-2.0 ml of standard BHA solution were transferred to the resulting solution and absorbance of the orange red colored chromogen were measured at 510 nm against the reagent blank.

### Method B:

Into a series of 25 ml calibrated tubes; FeCl<sub>3</sub> (1ml), 2,2-bipyridine (1ml) were taken. These tubes were kept in a hot water bath for 10min. at 100°C and then cooled to room temperature. To develop the initial color, Orthophosphoric acid solution(2ml) was added to each tube. Aliquots of 0.4-2.0 ml of standard BHA solution were transferred to the resulting solution and absorbance of the orange colored chromogen were measured at 520 nm against the reagent blank.

## RESULTS AND DISCUSSION

The results of analysis for method A and B were validated through systematic statistical analysis and results are tabulated. The statistical

analysis values are reported in Table -1 and assay and recovery results for this methods are tabulated in Table-2.

**Table 1.** Optical characteristics, precision and accuracy of BHA

Parameters	Developed method- A	Developed method-B
Name of the method	1,10-Phenanthroline, Ferric chloride	2,2-Bipyridine, Ferric chloride
$\lambda$ max(nm)	510nm	520nm
Beer's law limit( $\mu\text{g/ml}$ )	0.4-2.0	0.4-2.0
Sandell's Sensitivity ( $\mu\text{g/cm}^2/0.001$ abs. Unit)	0.026	0.038
Molar absorptivity	$0.684 \times 10^4$	$0.473 \times 10^4$
Correlation coefficient(r)	0.9998	0.9998
Slope(b)	0.04	0.003
Intercept(a)	4.310	0.018
Standard deviation	$0.012 \times 10^{-3}$	$0.010 \times 10^{-4}$
%RSD**	1.60	1.87
% Range of errors (confidence Limits):		
0.05 Significance levels	$\pm 1.338$	$\pm 1.564$
0.01 Significance levels	$\pm 1.979$	$\pm 2.313$

$Y = a + bx$ , where 'Y' is the absorbance and x is the concentration of BHA in  $\mu\text{g/ml}$ .

**Table 2.** Recovery of BHA in Biological Samples

Biological Sample (coconut oil) (ml/vial)	Amount of BHA added to biological sample	Amount found		% Recovery by developed method	
		Method-A	Method-B	Method-A	Method-B
Sample- 1	50 $\mu\text{g}$	46	45	92.00	90.00
Sample- 2	70 $\mu\text{g}$	64	66	91.42	94.28
Sample -3	90 $\mu\text{g}$	82	87	91.11	96.66

## DISCUSSION

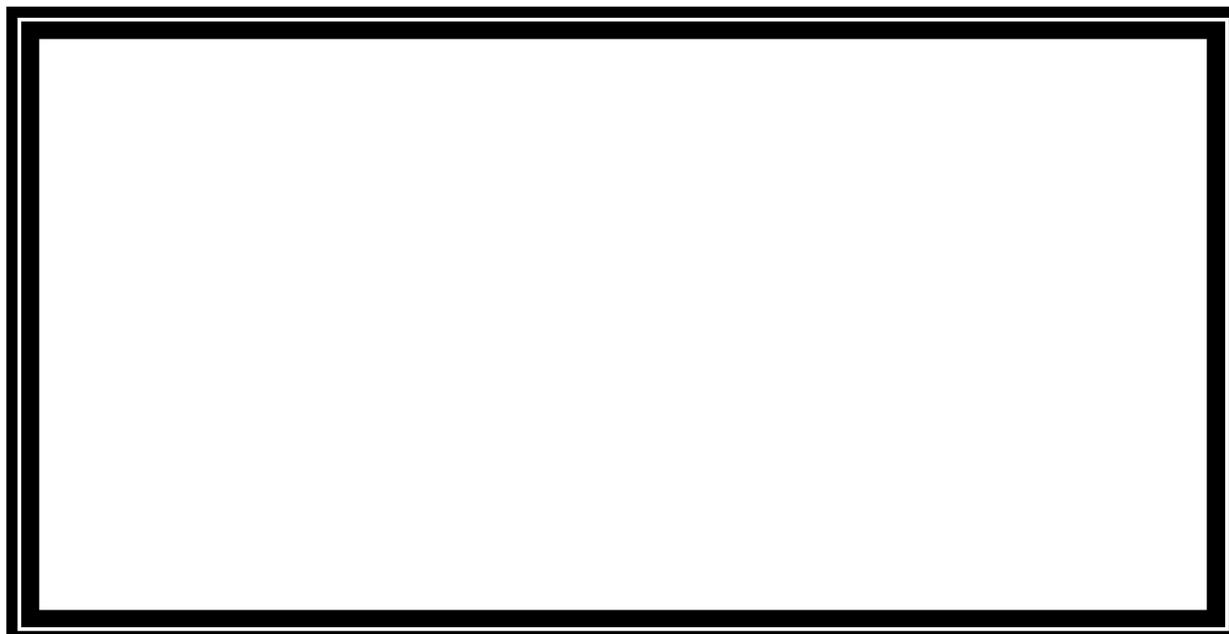
It deals about the mechanism of oxidation (method-A) and redox reaction (method-B) followed by complex formation

### Method A:

The proposed method is based on the mechanism of oxidation followed by complex formation, where in the initial reaction the anti-oxidant undergoes oxidation in the presence of ferric chloride and then the oxidized ferric

chloride reacts with 1,10- phenanthroline and the antioxidant BHA to form a orange red

colored complex which exhibits maximum absorption at wavelength of 510 nm.



**FIG 2:** Mechanism of reaction between BHA and 1,10- phenanthroline

**Method B:**

The results obtained in this method were due to redox reaction followed by complex formation between the anti-oxidant and ferric chloride and 2,2-bipyridine to form a orange colored

solution that exhibited maximum absorption at 520nm against the corresponding reagent blank.



**FIG 3 :** Mechanism of reaction between BHA and 2,2-bipyridine

For these methods optical characteristics such as absorption maxima, Beer's law limits, molar

absorptivity regression analysis using the method of least squares, slopes (a), intercept (b) and correlation coefficients (r) obtained from different concentrations are summarized in Table-1. The precision and accuracy were found by analyzing five replicate samples containing known amounts of the anti-oxidant and the results summarized in Table-1. The accuracy of these methods in the case of formulations was thoroughly studied by recovery experiments and the results were presented in Table-2. Additional checks on the accuracy of these methods were analyzed by adding known amounts of pure anti-oxidant to pre-analyzed formulations.

## CONCLUSION

Performance recovery experiments and percent recovery values obtained in this work indicated the absence of interferences from commonly encountered pharmaceutical additives and excipients. Though in earlier reported methods of analysis for BHA were not found, and hence for now this could be for now considered as a protocol for the estimation of BHA. The developed method is simple and sensitive with reasonable precision and accuracy and can be used as a standard method for the routine determination of BHA in quality control analysis.

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