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Spectroscopic Method for Determination of Butylated Hydroxyanisole (BHA)

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Abstract
Sophisticated analytical method viz. Spectrophotometric method which being employed for analysis, is relatively expensive and hence need for simple analytical method arises. In the proposed research work, such method have been developed and applied for routine determination of Butylated hydroxy anisole (BHA) in pharmaceutical formulations and bulk dosage forms. This method was based on the formation of colored species on binding of ferrous ions with MBTH in the presence of HCl, and the colored chromogen obtained was finally treated with the antioxidant BHA to produce green color with λ_{max} at 625 nm. Statistical analysis of this method exhibited Sandal's Sensitivity of 0.0264, and the relative standard deviation (RSD) of this method was found equal to 0.8733, indicating that this method is 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.*

INTRODUCTION

Butylated Hydroxy Anisole (BHA):

BHA ($CH_3C_6H_3(OH)C(CH_3)_3$) 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)[1]. BHA can induce allergic reactions in the skin (U.S. National Library of Medicine, (2010)) [2]. 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))[3]. 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))[4].

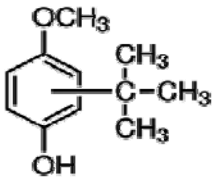

FIG 1: Structure of BHA

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))[5]. It can cause or aggravate conditions such as asthma and hives, as well as developmental disorders in children (Lahey, M., Rosen, S., (2007)) [6]. 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)) [7].

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)) [8]. 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_2), 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_9 mix. In some instances the substances were unstable in the test procedure (Kalus WH (1990)) [9].

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)) [10]. 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)) [11].

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.

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:

- MBTH (0.2% W/V)
- $FeCl_3$ (0.7% W/V)
- HCl (0.5N)
-

ASSAY PROCEDURE

Into a series of 25 ml calibrated tubes; Aliquots of 0.4-2.0 ml of standard BHA solution were taken. Millipore double distilled water was added to make the volume to 2 ml. 0.5 ml of HRP (Horse raddish peroxidase) enzyme was added to each tube. These tubes were kept for 10min. at room temperature. 1ml MBTH is added to each tube and kept for incubation for 5 min. at room temperature. 0.5 ml $FeCl_3$ is added to each tubes and again kept for incubation for 5 min. at room temperature. And finally 0.5 ml HCl is added to each Aliquots where green colour is developed and absorbance of the green colored chromogen were measured at 625 nm against the reagent blank.

RESULTS AND DISCUSSION

The result of analysis for method was validated through systematic statistical analysis and results are tabulated .The statistical analysis values are reported in Table -1.

Discussion

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 MBTH and the antioxidant BHA to form a green (scheme 1) colored complex which exhibits maximum absorption at wavelength of 625 nm.

For the method 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.

Table 1. Optical characteristics and precision of BHA

Parameters	Developed
Name of the method	MBTH, Ferric chloride
λ max(nm)	625 nm
Beer's law limit(µg/ml)	0.4-2.0
Sandell's Sensitivity (µg/cm 2/0.001 abs. Unit)	0.0264
Molar absorptivity	0.6804
Correlation coefficient(r)	0.9999
Slope(b)	0.00333
Intercept(a)	0.00016
Standard deviation	0.00229
%RSD**	0.8733
% Range of errors (confidence Limits):	
0.05 Significance levels	±1.338
0.01 Significance levels	±1.979

Y= a+bx, where 'Y' is the absorbance and x is the concentration of BHA in µg/ml.

Table 2. Results of analysis of tablet formulation by proposed method

Formulation	Label claim (mg/tab)	Amount found* mg/tablet ± SD	Percentage of label claim ± SD
Tablet 1	10	9.99 ± 0.202	99.79 ± 0.8087
Tablet 2	10	9.81 ± 0.772	99.25 ± 0.777

* 6 Replicates

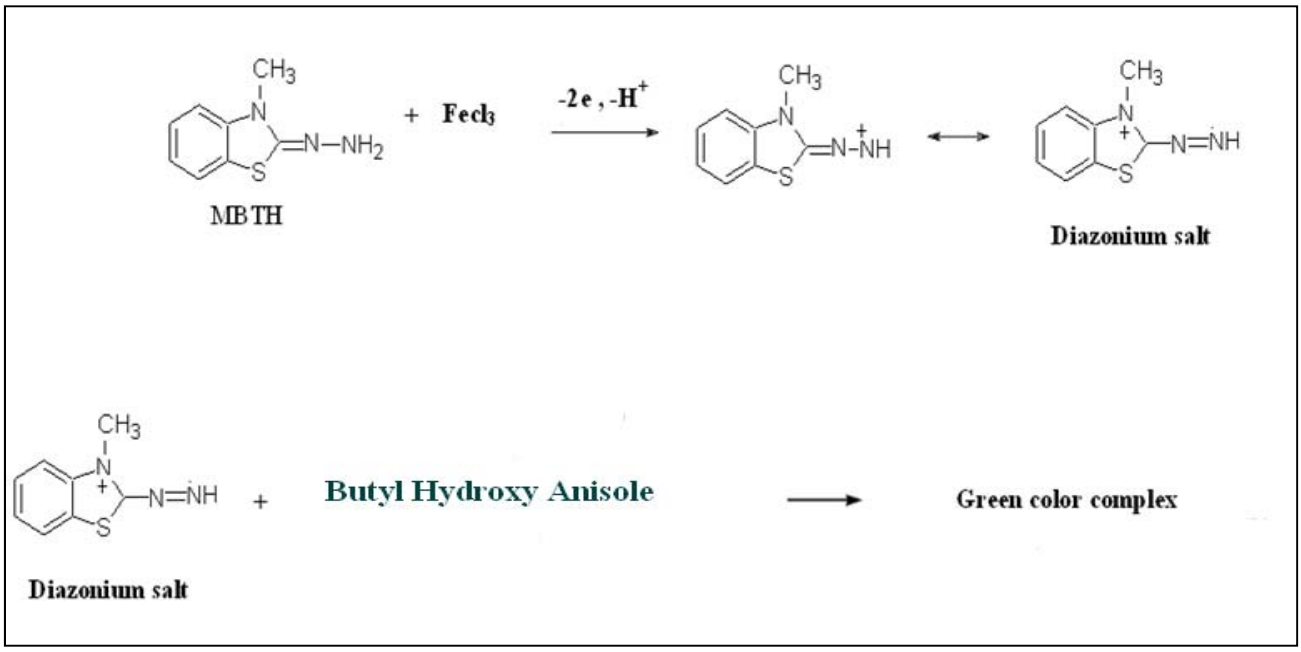

FIG 2: Reaction Mechanism

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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