J. Mountain Res. P-ISSN: 0974-3030, E-ISSN: 2582-5011 DOI:https://doi.org/10.51220/jmr.v17i2.4
Vol. 17(2), (2022), 35-38

Determination of Antioxidant (TBHQ) in Haldi liquid by High Performance Liquid Chromatography

Bhaskar C. Dwivedi[1*] • A. Pasrija[1] • R. Mitra[1] • P. Bandyopadhyay[1]
[1]*Dabur Research & Development canter, Dabur India Limited, 22, Site-IV, Sahibabad, UP, India*
*Corresponding Author Email- Bhaskar.chandra@dabur.com

Received: 02.12.2022; Revised: 21.12.2022; Accepted: 24.12.2022

Abstract: For increasing the self-life of packaged, Frozen & processed food products TBHQ plays a vital role in human life. FDA {FOOD and DRUG ADMINISTRATION} declared its intended use in 1972 so increasing the popularity worldwide. TBHQ- There are various Antioxidant which is used as food additives in food and Cosmetic products. TBHQ has a tendency to decrease the rancidity by preventing the oxidation reactions in the food meal. TBHQ are commonly used antioxidants. TBHQ are very much popular additives used as a preservative Therefore, our organisational research & development team developed new Ayurveda product for curing the human health disease like digestive disorders and depression and as well as increase the immunity. Analytical HPLC method needs to measure the antioxidants in the Haldi/Turmeric (*Curcuma longa* L.) Liquid. As there are so many methods to detect TBHQ in different type of new product formulations. In our R&D facility we developed and validated new HPLC {High Performance Liquid Chromatography} method of analysis for TBHQ in the Haldi liquid sample.

Keywords: Antioxidants • FDA • TBHQ • HPLC • RSD • Haldi liquid.

Introduction

Generally, the antioxidant t-butyl hydroquinone (TBHQ) is used as a food preservative in Food, Cosmetic and beverages. TBHQ prevents food and oil-containing products from rancidity. Actually, the process mechanism of TBHQ is able to inhibit the oxidation of a substance that is easily oxidized even in low concentrations. Analysis of TBHQ has been determined by HPLC quantitatively in Haldi/Turmeric (*Curcuma longa* L.) liquid. Haldi liquid is oil-based composition. TBHQ is used as a preservative agent in the sample. Although there are many methods of analysis to determine TBHQ (Razali et al 1997, Christinawaty 2015). But this method is more specific, precise, sensitive, accurate and reproducible for Haldi liquid sample analysis than the other traditional methods. This method is relatively easier to perform and precise for this compound. A reverse phase gradient chromatographic method has been developed at 200 ppm level in Haldi liquid sample.

Chemical Formula: $C_{10}H_{14}O_2$
IUPAC NAME: 2-(1, 1-Dimethyllethyl)-1, 4-benzenediol)

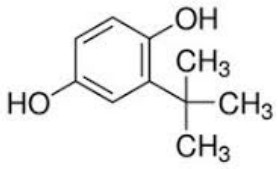

Fig 1. Chemical structure of TBHQ

Experimental Section

Materials and Methods

The High-Performance Liquid Chromatography (HPLC) was used from (Shimadzu) Model Prominence Quaternary pump (LC-2030C 3D) equipped with a 20 µl sample loop. Shimadzu PDA detector was carried out on column (250 x 4.6 mm i.e.) packed with Octadecyl dimethyl packing. TARSON TUBE, SPINIX (VORTEX SHAKER) 220V, 50Hz. Calibrated volumetric flask and glassware used was rinsed with

acetone before use. Sample was in liquid form and stored in an airtight container at 25°C.

Reagents

Solvents used in the experiment**,** *viz.* methanol, milli-q-water HPLC grades from Reference standard, TBHQ (Sigma-Aldrich)

A solution of acetonitrile contains 1% acetic acid (Solvent A) and a solution of milli-q-water contains 1% acetic acid (Solvent B)

Gradient

Time(min)	Solvent A	Solvent B
0.01	30	70
7.0	30	70
12.0	100	0
15.0	30	30
16.0	30	70

Methods Specificity

The specificity of the method was monitored by analysing the placebo and standard solution. There was no peak close to the retention time of TBHQ, which proved the high degree of specificity of the method. As shown in Figure-1.

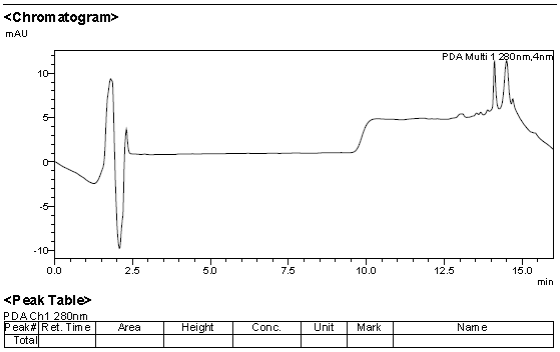

Fig.1

Reference Standard solution

Standards of TBHQ carefully weighed approximately 12 mg inserted into 100 mL volumetric flask, add 50 ml methanol sonicate to dissolve make up volume up to the mark. Standard concentration is 120 µg/mL. The solution was filtered using a membrane filter of 0.45 µm PTFE. Peak purity, spectra of the peak and standard solution chromatogram shown in figure-2,3 & figure-4.

System suitability

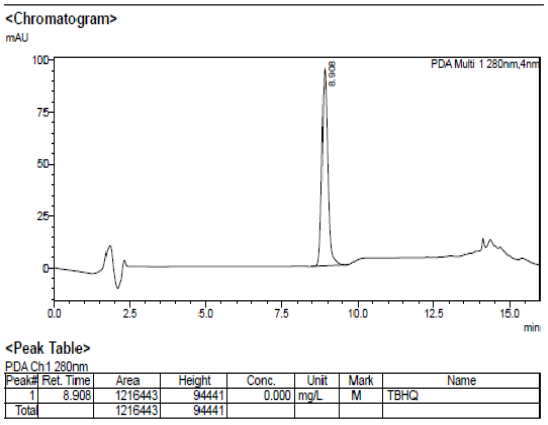

Fig. 2: Chromatogram of standard solution (TBHQ: Tertiary butyl hydroquinone**)**

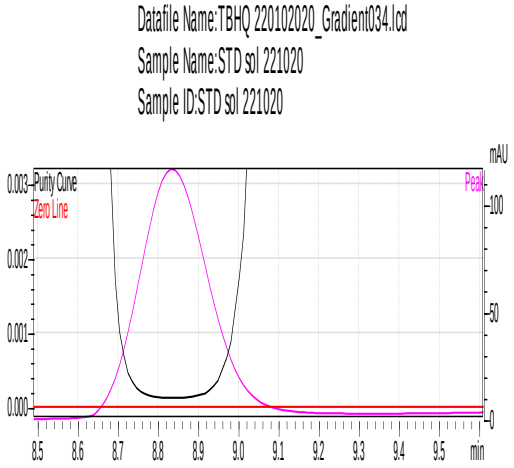

Fig. 3: Peak purity chromatogram of standard (TBHQ: Tertiary butyl hydroquinone**)**

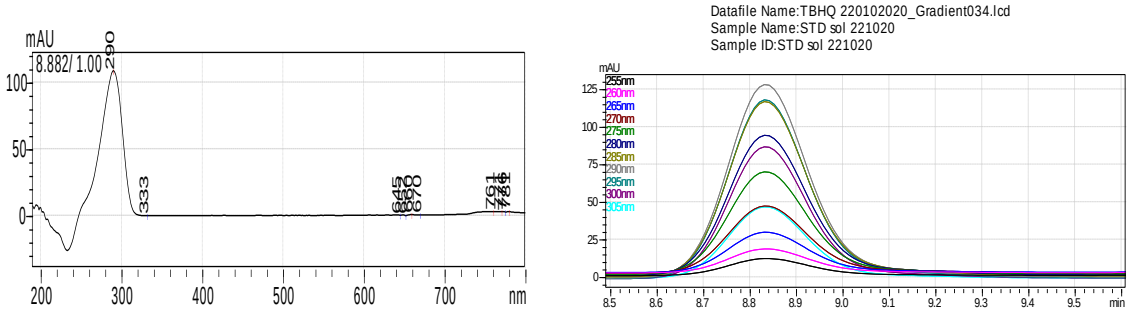

Fig. 4: Spectra of standard solution (TBHQ: Tertiary butyl hydroquinone**)**

Results

Result of system suitability is shown in Table 1 whereas the results of the validation of the HPLC method including standard chromatogram, peak, purity, spectra, linearity, limit of detection and quantitation limits, accuracy and precision are shown in Figures: 1, 2 and 3 and Tables 1, 2. Moreover, the determination of sample is (n=3) repetitions is shown in Table 3.

Method validation criteria

1. Specificity
2. Recovery
3. Linearity
4. Limits of detection and quantification
5. Quality of data (accuracy and precision)
6. Ruggedness

Accuracy

The closeness between the expected value and the final value found as a result, depends on the accuracy of an analytical method. It is obtained after calculating the percent recovery (%R) of the analyse recovered. In this study, to evaluate the accuracy of the developed method, successive analysis (n = 3) for three different concentrations (20 mg.ml^{-1}, 25 mg.ml^{-1} and 30 mg.ml^{-1}) of standard TBHQ solution were performed using the developed method. The data of the experiment were statistically analysed using the formula [% Recovery = (Recovered conc. /Injected conc.) × 100] to the study the recovery and validity of the developed method. The mean recovery should be within 90–110% to be accepted.

Recovery of antioxidants (TBHQ) added to haldi liquid

At 80% level by using 20 ppm added

Antioxidant	Found (ppm)	Recovery (%)	%RSD
TBHQ	19.1	95.5	1.92
TBHQ	18.5	92.5	1.92
TBHQ	18.4	93.3	1.92

At 100% level by using 25 ppm added

Antioxidant	Found (ppm)	Recovery (%)	%RSD
TBHQ	23.9	95.6	1.12
TBHQ	24.1	96.4	1.12
TBHQ	24.4	97.6	1.12

At 120% level by using 30 ppm added

Antioxidant	Found (ppm)	Recovery (%)	%RSD
TBHQ	28.6	95.3	0.94
TBHQ	29.1	97.0	0.94
TBHQ	29.0	96.6	0.94

Linearity

Linearity done at 5,10,15,25 and 50 ppm level

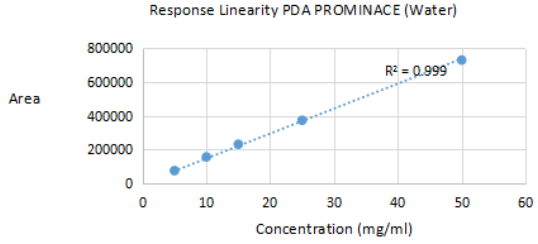

Fig. 5: Linearity curve of (TBHQ: Tertiary butyl hydroquinone**)**

Table 1: Determination system suitability test of TBHQ

Standard	Theoretically plate number (N)	Symmetry factor
TBHQ	195224.35	1.13

TBHQ: Tertiary butyl hydroquinone

Table 2: Value of r Detection (µg/ml) and Limit of Quantitation (µg/ml) of TBHQ

Compound	r^2	LOD (µg/mL)	LOQ (µg/mL)
TBHQ	0.9998	1.118	2.446

TBHQ: Tertiary butyl hydroquinone, LOD: Limit of detection, LOQ: Limit of quantitation

Table 3: Intra-day and Inter-day Precision of TBHQ (µg/ml)

TBHQ Concentration (µg/ml)	Intra-day %RSD	Inter-day %RSD		
		1st Day	2nd Day	3rd Day
10	1.64	0.98	1.70	1.04
15	1.28	1.34	1.50	1.65
25	1.80	1.80	1.83	1.52

TBHQ: Tertiary butyl hydroquinone, RSD: Relative standard deviation

The mobile phase used in the analysis of TBHQ was a mixture of 1% acetic acid in acetonitrile and 1% acetic acid in milli-q-water. Analysis done in the gradient phase. About 1.0 g of Haldi liquid sample was accurately weighed that have been homogenized and previously shake transferred into the 50 ml volumetric flask and add 25 mL of methanol, keep the sample on the sonicator up to 15 minutes makeup volume up to the mark on the volumetric flask. Sample was filtered with a membrane filter of 0.45 µm PTFE, HPLC analysis by using PDA (Photo Diode Array) detector at a wavelength of 280 nm. Determination of TBHQ in three repetitions. Validation of the HPLC method includes linearity, LOD9Limit of detection), and quantitation limits (LOQ), accuracy and precision.

Conclusion

HPLC chromatographic technique's is the best suitable methodology to detect TBHQ in Haldi/Turmeric (*Curcuma longa* L.) liquid and it's kind of products. Method was expressed as RSD 1.7 and recovery 96.4% method was found to be simple, and robust for the analysis of Haldi liquid sample. The results obtained are shown in the process are specific accurate and precise. The procedure is found very short and rapid for general analytical use of the HPLC chromatography quantification purpose.

Acknowledgements

We are grateful to our organization team of Dabur analytical research & development, Sahibabad, UP to provide facilities for the research work and their support time to time.

References

Razali, L., Norhaya, H. Annnor, Asimah, A.S. (1997). Determination of Antioxident in Palm oil products by High performance liquid chromatography. Elaeis Vol. 9(1): 25-33.

Christinawaty, Sophi, D. (2015) Simultaneous determination method of butylhydroxyanisole, butyl hydroxyl toluene, Propyl gallate, and Tertiary butyl hdroquinone in margarine using High performance liquid chromatography, Asian Jurnal of Pharmaceautical and clinical research. 8(4): 209-211.