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APPLICATIONS OF MBTH AS A CHROMOGENIC REAGENT: A REVIEW

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ABSTRACT

For quantitative and qualitative analysis of pharmaceutical medicinal substances, contemporary and extensive colorimetric indicators were used. Functional groups in pharmaceutical drug substances aid in drug substance estimation. This review summarizes the applications of MBTH (3-methyl -2- benzothiazolinonehydrazone hydrochloride) as chromogenic reagents for spectrophotometric analysis of pharmaceutical compounds. This reagent has many applications in the pharmaceutical industry and is adaptable to various analytical techniques.

Keywords, MBTH, Spectrophotometry, Oxidative coupling, Applications

INTRODUCTION

MBTH (3-methyl -2- benzothiazolinonehydrochloride) is an extensively used variable chromogenic reagent in colorimetric analysis of pharmaceutical drug substances in analytical, biochemical, and synthetic procedures. Besthorn first manufactured MBTH reagent in 1910. Huning and Fritsch explained the oxidative coupling reaction of MBTH with phenols, aromatic amines, heterocyclic bases, and compounds containing methylene groups in 1957. Using this fact, Sawicki popularized MBTH for quantitative analysis of carbonyl compounds **[1]**. This prompted MBTH to broaden its application to the colorimetric estimation of a variety of organic compounds and pharmaceuticals containing phenols, aryl amines, and aromatic amines. When used as an aqueous solution, the reagent produces chromogen or a colored substance **[2]**. In addition, it is used to determine ethylenic compounds, primary alcohols, glycols, glycerides, carbohydrates, vitamins, and steroids after previous reactions such as oxidation, reduction, and hydrolysis **[3]**.

The chemical formula of the MBTH reagent is (*E*)-(3-methyl-1,3-benzothiazol-2-ylidene) hydrazine; hydrochloride **(Figure 1)**. Other synonyms for it include Besthorn hydrazone. Sawicki's reagent, MBTH. It is a white to off-white substance with a melting point of 276-278ºC and a boiling point of 342ºC. It has a molecular weight of 233.718 and the formula $C_8H_{12}ClN_3S$.

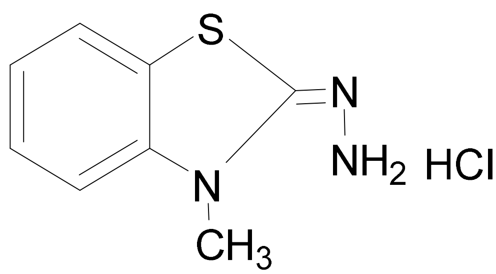

Figure 1: Structure of MBTH

Principle

Oxidation was followed by a coupling reaction in accordance with the concept.Oxidant catalyzes an oxidative coupling reaction in MBTH. Ferric Chloride, Cerric Ammonium Sulfate, Potassium dichromate, Potassium bromate, Ammonium per Sulfate, etc. are the most frequently used oxidants for the formation of color species. Under the conditions, MBTH loses two electrons and one proton, forming an electrophilic intermediate that is an active coupling agent. This intermediate undergoes electrophilic substitution with phenols, amines, and aldehydes to form chromogen **[4, 5]**. **Figure 2** depicts the typical reaction to MBTH.

Mechanism

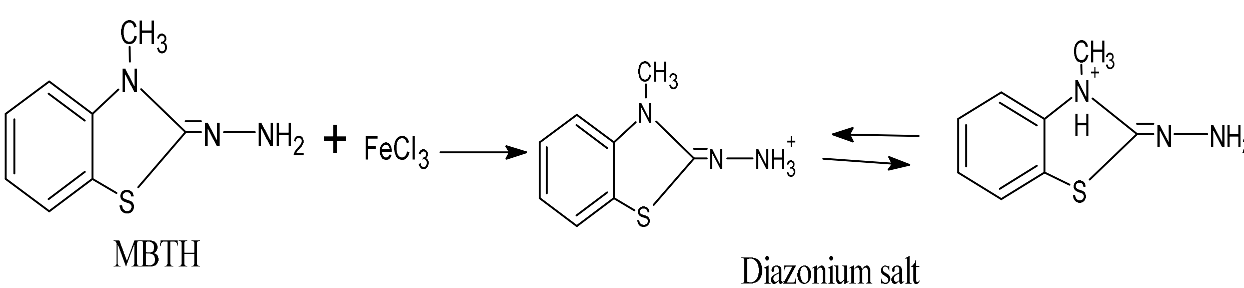

Figure 2: General reaction with MBTH

Applications

Estimation of drug substance in biological samples (blood and urine)

Determination of total estrogens in urine with MBTH method has been evaluated **[6]**. Colorimetric measurements were made at 530 nm or by using auto analyzer at rate of 50 samples per hour. 0.2%w\v ceric ammonium sulphate in 2% v\v H_2SO_4, 0.15% w\v MBTH, 0.3% w\v EDTA solutions were used.

Ramdzan *et al*. **[7]** reported the use of gas diffusion flow injection analysis for the determination of acetaldehyde in saliva.

Identification of aldehyde, amines, phenols, aryl amines

According to Archer's findings, the analysis of phenolic lichen compounds can be conducted through the utilization of MBTH as a spray reagent **[8]**. Lichens are capable of synthesizing lichen acids, which are phenolic carboxylic acid derivatives. These compounds can be effectively isolated through thin layer chromatography (TLC) and subsequently detected using the MBTH spraying reagent.

The performance of carbonyl compounds' 3-methyl-2-benzothiazolone azines in high-performance liquid chromatography (HPLC) was reported by Chiavari and Facchini **[9]**. The MBTH derivatization method was utilized in the primary phase to produce azine compounds, which were subsequently identified via electrochemical modalities.

Chiavari **[10]** developed a method for the high-performance liquid chromatography (HPLC) analysis of aldehydes at low concentrations through the use of their 3-methyl-2-benzothiazolinone hydrazone (MBTH) derivatives.

A study showcased an enhanced TLC technique for the identification and detection of cannabinoids in cannabis **[11]**.

In their study, Setti *et al*. presented a technique involving the use of laccase as a catalyst for the oxidative coupling of MBTH and methoxyphenols **[12]**. The present study describes a reaction that entails the oxidative coupling of MBTH and o-, m-, and p-methoxyphenols using O_2 as the oxidizing agent.

Using MBTH as a derivative agent, Ledauphin *et al*. devised a GC quantification of aliphatic aldehydes in newly distilled calvados and cognac **[13]**. GC-MS was used to isolate and identify carbonyl compounds after they had been derivatized using MBTH. To achieve a good sensibility (0.2–1.2 μg/L) and a fair selectivity, selective mass spectrometric detection of molecular ions of derivatives was carried out.

Chiavari and Facchini **[14]** described how 3-methyl-2-benzothiazolone azines of carbonyl compounds behaved in HPLC. The column's operating parameters include a mobile phase of methanol and KH_2PO_4, a 20µl injection volume, etc. Here, the first step of the MBTH derivatization process was used to create azine chemicals, which were then identified electrochemically. (by evaluating peak heights).

Glyoxal in aldehyde solutions may now be determined using HPLC and MBTH **[15]**. The column settings included a 0.8 ml/min flow rate, a 10 µl/min injection volume, and a 95,5 acetonitrile,water mobile phase. The produced glyoxal diazine had a retention duration of 5.2 minutes and was a yellow dye with a maximum wavelength of 401 nm.

To evaluate the phenolic compounds by optical technique, MBTH was stacked on film immobilized in hybrid nafion/sol-gel silicate film and horseradish peroxidase (HRP) in chitosan **[16]**.

Analysis of Environmental samples

The MBTH method for determining phenols in water and waste fluids was proposed for study by Gales *et al*. **[17]**. Velikonja *et al*. **[18]** suggested comparing GC and spectrophotometric methods for figuring out how much formaldehyde is in a given amount of water.

HPLC can be used to investigate the phenolic chemicals in industrial effluent, and post-column reaction detection has been described **[19]**. The column conditions included flow rate (0.7 ml/min), injection volume (2–50 µl), mobile phase composition of acetonitrile (95), water (5), etc. Two post-column reagents are 0.15% aqueous MBTH-HCl solution and 0.4% Ce $(NH_3)_2(SO_4)_3$ solution in 5% v/v H_2SO_4.

A proposed method for spectrophotometric determination of residual chlorine in drinking water and environmental water samples involves novel oxidative electrophilic coupling reactions of phenoxine derivatives with MBTH, as reported in the literature **[20]**. There have been reports of a highly sensitive reaction of nitrate with brucine and MBTH for the determination of nitrate in environmental samples **[21]**. At 560 nm, a violet-colored product was produced when nitrate reacted with brucine-MBTH. In an acidic medium, nitrate reacts with brucine only at 410 nm to form a yellow-colored product.

Suvardhan *et al*. **[22]** proposed a spectrophotometric method for determining the concentration of tellurium in environmental and telluride film samples. Based on the oxidation of MBTH by tellurium in a basic medium and coupling with chromotropic acid, a red-colored species was produced. Beer's law was observed between 0.7 and 20 µg/m1.

Vanadium may be easily and accurately determined by spectrophotometry in a variety of materials, including soil, water, urine, alloy steels, and biological and pharmaceutical substances **[23]**. At 595 nm (blue color) and 526 nm (pink color), absorption maxima were discovered.

Determination of Antioxidants

The process of determining certain antioxidants, namely tertiary butyl hydroquinone (TBHQ), butylated hydroxyl anisole (BHA), and gallic acid (GA), in oils and fats through spectrophotometry has been documented in the literature **[24]**. This method involves the utilization of the MBTH reagent and ceric ammonium sulfate. The maximum wavelengths recorded for TBHQ, BHA, and GA were 500 nm, 480 nm, and 440 nm, respectively.

Determination of proteoglycans

For the quantitative analysis of nonenzymatically glucosylated proteins, the sialic acid-MBTH assay was modified **[25]**. It involves glucosylated proteins being subjected to periodate oxidation at pH 3.0 for five minutes, producing formaldehyde. When it complexes with the MBTH reagent, it is then identified.

Determination of pharmaceutical drug substances

It has been shown that various local anesthetics (benzocaine, procaine HCl, and amethocaine HCl) can be estimated spectrophotometrically using MBTH **[26]**. At 575 nm (benzocaine and procaine) and 615 nm (amethocaine), the blue color chromogen was obtained.

Michael E. El-Kommos proposed using MBTH as a chromogenic reagent for the spectrophotometric analysis of several sulpha pharmaceuticals **[27]**. The MBTH solution utilized was 0.35% w/v.

El-Yazbi *et al*. **[28]** proposed a spectrophotometric method for determination of benzapril hydrochloride in its single and multi-component dosage forms

Using 0.4% wv of MBTH, a spectrophotometric method was devised for the determination of antidepressant drugs such as amitriptyline hydrochloride, nortriptyline hydrochloride, and doxepin hydrochloride in pure and dosage forms **[29]**.

El Ragehy *et al*. **[30]** developed a stability-indicating method for the determination of nortriptyline hydrochloride using MBTH.

Sastry C S P *et al*. developed a method for determining the concentration of ritodrine hydrochloride in bulk samples and dosage forms **[31]**.

Dias *et al*. **[32]** reported a multi-pump flow system for the spectrophotometric determination of bromhexine.

For the purpose of identifying caffeine and theophylline in pure alkaloids and their uses in pharmaceutical formulations, Singh D. K. created a spectrophotometric method **[33]**. Caffeine and theophylline were oxidized using sodium metaperiodate in the presence of acetic acid, then coupled with MBTH to create a blue product with an absorption maximum at 630 nm.

For the development and validation of LC techniques with visual detection, Raman *et al*. proposed utilizing mass detection and pre-column derivatization for the assay of voglibose (VB) **[34]**. After being derivatized using sodium metaperiodate and MBTH, VB was passed through a column containing a mobile phase of acetonitrile and pH 6.0 buffer (35,65 v/v).

Using the oxidative coupling reaction of MBTH, Ribeiro *et al*. **[35]** devised a method for determining indapamide. Utilizing a multipumping flow system, 1.2×10^{-2} mol\L of MBTH solution and 1.1×10^{-2} mol\L of Ce (IV) solution were used.

Chandan *et al*. **[36]** proposed a spectrophotometric method for measuring lamotrigine (LTG) in pharmaceutical dose forms. The process was predicated on the fact that LTG reacts with MBTH to produce a green product that can be seen at 662 nm.

Kalyanaramu *et al*. developed a method for raloxifene hydrochloride determination by treating the substance with MBTH reagent in the presence of ferric chloride at λ_{max} of 649.3 nm **[37]**.

Using 0.5% w/v MBTH, a spectrophotometric method was devised for the estimation of pregabalin (PGB) in its dosage forms **[38]**. The chromogen exhibits maximal absorption at 668 nm and was stable for two hours.

Shravya *et al*. proposed a spectrophotometric method for determining ezetimibe in pharmaceutical dosage form using MBTH reagent **[39]**.

Bab *et al*. **[40]** performed an assay of tolterodine tartrate (TT) using MBTH reagent in bulk and tablet dosage forms. TT reacts with MBTH reagent in the presence of $FeCl_3$ to produce a blue compound at λ_{max} 650 nm.

Kutty *et al*. **[41]** described a validated UV-visible spectrophotometric method for the estimation of fenofibrate in bulk drugs and dosage forms. Using solutions such as 0.5% MBTH and 1% $FeCl_3$ in 0.5% HCl, the absorbance was measured at 596 nm.

The quantitative spectrophotometric measurement of oxcarbazepine (OXC) in pharmaceuticals was developed using cerium (IV) and periodate **[42]**. The oxidative coupling of OXC, MBTH, and cerium (IV) sulfate at pH 4.28 ± 0.07 or sodium periodate at pH > 4.0 resulted in an orange-colored molecule at λ_{max} 450 nm.

Using the MBTH reagent, a straightforward and sensitive spectrophotometric method for the determination of risperidone has been described **[43]**. At λ_{max} 595.8 nm, the apple green color chromogen is formed. Beer's law was observed to hold true over a concentration range of 10-80 µg\ml, and a regression coefficient of 0.99 was determined.

CONCLUSION

Recently, significant advancements have been made in the utilization of MBTH for the creation of spectrophotometric techniques aimed at detecting aldehydes, phenols, and amines. The pharmaceutical formulations have been utilized to determine the drugs quantitatively in both pure form and commercial preparations. This review article provides a comprehensive overview of the MBTH reagent and its various applications. It is intended to enhance the reader's understanding and facilitate the development of suitable analytical methods for pharmaceutical drug substances.

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