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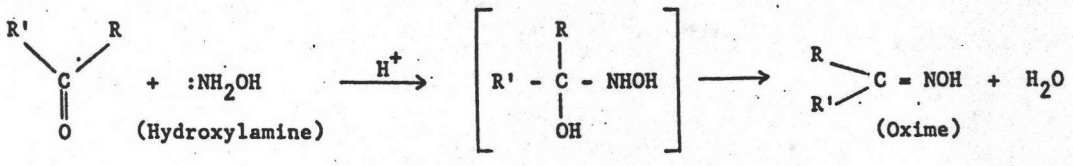
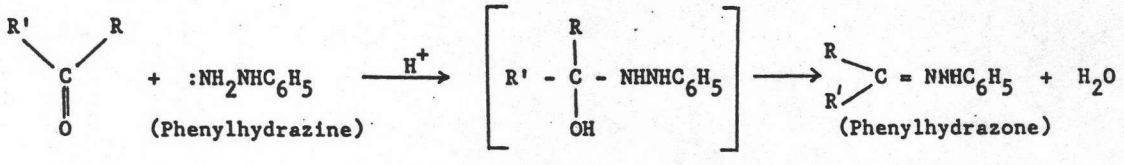
CHAPTER I

INTRODUCTION

2,4-Dintrophenylhydrazine (2,4-DNPH) has been used to characterize and identify many aldehydes and ketones for many years (1-3). The reaction of aldehyde or ketone with 2,4-DNPH is nucleophilic addition reaction (4). 2,4-DNPH is a nucleophile with a basic nitrogen that can attack carbonyl carbon of aldehydes or ketones. The reaction medium must be acid like other derivatives of ammonia because the first step of mechanism of reaction is protonation of carbonyl group by proton [H^+] from acid (5). (Figure 1)





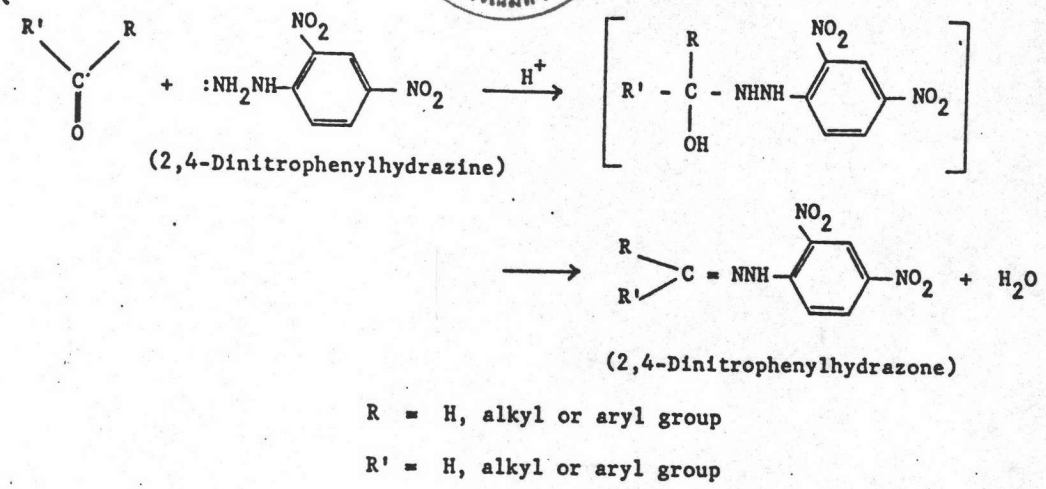
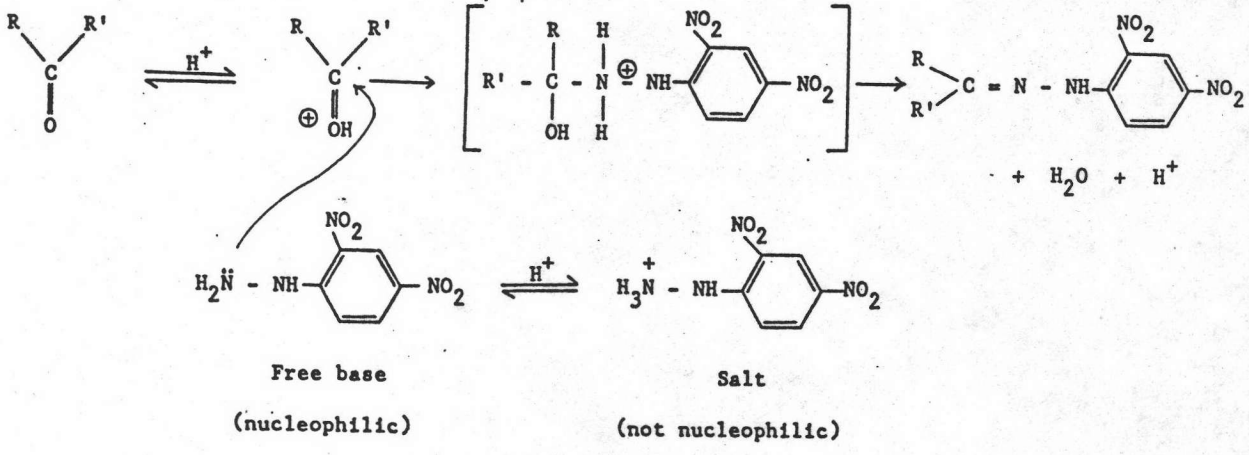


Figure 1 Nucleophilic addition reaction of carbonyl compounds

The acidity of the reaction must be justified in order to protonate the carbonyl oxygen and make carbonyl carbon more susceptible to nucleophilic attack. 2,4-DNPH is also protonated to form an ion which lacks unshared electrons and is no longer nucleophile.



The optimum acidity should compromise between the protonation of carbonyl group and the forming of free base of 2,4-DNPH.

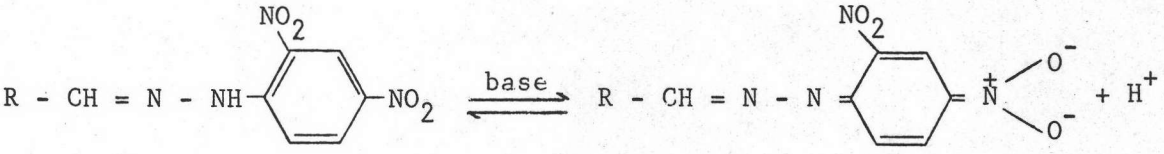
Ketone or aldehyde reacts with 2,4-DNPH to yield a yellow to orange-red precipitate of 2,4-dinitrophenylhydrazone (2,4-DNPH' zone) derivative. The UV-absorption spectra of 2,4-DNPH' zone derivatives were studied (6,8). The absorption spectra are mostly based on the structure of the parent carbonyl compounds. Substitution of aliphatic or aromatic functional groups on parent compounds cause variation in maximum absorption wavelength and/or absorption intensity. 2,4-DNPH' zone formation is also the basis of a good gravimetric determination of carbonyls because it is almost insoluble in acid solution (9).

I UV-V Spectrometric Analysis of Carbonyl Compound

Many UV-V spectrometric analyses of carbonyl compounds were based on 2,4-DNPH' zone formation. M.F. Pool (10) determined mono-carbonyl compounds in the benzene-soluble fraction of rancid foods by forming 2,4-DNPH' zones. Removing of unreacted excess reagent with alumina, and colorimetric measurement of the remaining 2,4-DNPH' zone in alkaline solution can be performed. M. Pesez (11) determined carbonyl compounds by forming orange color of 2,4-DNPH' zone in acetic-hydrochloric acid medium at room temperature. Under such conditions the blank reading is negligible. Some carbonyl compounds and steroids had to be used high temperature in order to form such derivatives.

G.R. Lappin and L.C. Clark (12) determined traces of carbonyl compounds by adding a solution of sodium or potassium hydroxide to an

alcoholic solution of a 2,4-DNPH' zone. The result was a very intense wine-red color solution due to the formation of the resonating quinoidal ion.



quinoidal ion

F.K. Critchfield and J.A. Hutchinson (13) determined secondary alcohols which were firstly oxidized with acidic potassium dichromate. The amount of ketones forming in the reaction was determined by 2,4-DNPH'zones formation. F.H. Lohman (14) determined carbonyl compounds by a method based on 2,4-DNPH' zone formation. The yellow products were separated from excess reagent by extracting with hexane. The spectra of the pure, neutral 2,4-DNPH' zones were very reproducible and the colors were stable. P.E. Toren and B.J. Heinrich (15) determined butadiene-furfural condensation product [2,3,4,5-bis (2-butenylene) tetrahydrofurfural] by reaction of the compound with 2,4-DNPH in a two-phase system, composed of iso-octane and an alcohol -water-phosphoric acid mixture. The 2,4-DNPH' zone was selectively extracted into the iso-octane phase which was used for spectrometric analysis.

II Analysis of Mebendazole

Many methods have been used to assay mebendazole in pharmaceutical preparations and raw material. Some of them are described below.

1. Non-Aqueous Titration

Mebendazole contains a basic side chain at carbamate ester, so it can be titrated with perchloric acid quantitatively. The non-aqueous titration procedure was a suitable method for both raw material and mebendazole tablets (16,17). The non-aqueous medium was a mixture of a few mls of formic acid and glacial acetic acid, or only glacial acetic acid which must be warmed to dissolve mebendazole. Crystal violet was a suitable indicator for use in both direct and indirect titration. For direct titration, perchloric acid was used (16,17). For indirect titration (17), an excess of perchloric acid was added, and the excess was titrated with sodium acetate in acetic acid.

The non-aqueous titration is the method which is prefered to use in the preparations containing coloring agent or other additives which may interfere the photometric method.

2. Photometric Method

2.1 UV-Spectrometric Method

UV-spectrometric method is widely used for determining many drugs. Mebendazole contains chromophores that can absorb UV-light, so the assay using UV-spectrometric method can be applied. Selection for proper solvent is also important in any assays of mebendazole and its pharmaceutical preparations. For examples, mebendazole tablets could be determined by using 70% perchloric acid as solvent and the proper concentration was made with purified water. The measurement of absorbance at 288 nm was performed in order to assay the content (17).

The USP method (18) for determining mebendazole in tablets was UV-spectrometric measurement at the maximum absorbance wavelength of 247 nm after extracting mebendazole with proper solvent.

2.2 Colorimetric Method

Colorimetric method is one of the specific functional group analysis of drugs. A.A. Patel et al. (19) had reported the determination of mebendazole in pharmaceutical preparation by using formic acid as solvent, and adjust to suitable concentration with isopropyl alcohol. An aliquot of the sample preparation was treated with methanol and potassium hydroxide solution respectively, and allowed this mixture to stand for 30 minutes for the color developing. The absorbance was measured at 420 nm against the reagent blank and the concentration of the sample preparation was determined. This method was based on alkaline hydrolysis of mebendazole to 2-amino-5-benzoyl-benzimidazole which gave the color reaction. To obey Lambert-Beer's law, the limit of concentration of mebendazole was 25-250 mcg/ml.

A. Kar (20) proposed the method which based on the formation of color complex of potassium bismuth (III) iodide and mebendazole. The sample of mebendazole was firstly extracted with water to remove water soluble matter. The remaining matter was dissolved in a mixture of formic acid and water, and mebendazole was precipitated with potassium bismuth (III) iodide solution. An orange precipitate, after washed with nitric acid to remove an excess reagent and dried, was redissolved in acetone and measured the absorbance at 430 nm. An optimum final concentration range which obeyed Lambert-Beer's law was 0.3 - 2.1 mg percent.

The colorimetric analysis of mebendazole can also be done by the reaction of hydroxyl-ammonium chloride, dicyclohexy-carbodiimide and ferric chloride in mixture of formic acid and isopropyl alcohol. The complex color formation was measured absorbance at 520 nm against blank, and the amount of mebendazole could be calculated on the basis of Lambert-Beer's law. The optimum concentration range which gave a good result was 0.24 - 2.0 mcg/ml (21).

2.3 Phosphorescence Method

Phosphorescence method for determining mebendazole was proposed by F.A. Fattah (22). The procedure was proceeded on Whatman 42 filter-paper and a self constructed sample holder designed for using with samples at room temperature. However mebendazole showed a good phosphoresence signal at 77°K.

To obtain a good result in measuring phosphorescence at room temperature, Pb (IV) and Tl (I) was recommended to use as a reagent to enhance phosphorescence, and the result indicated that Pb (IV) gave the highest emission intensities.

2.4 Fluorimetric Method

Fluorimetric method was proposed by F.A. Fattah et al. (23). It was a selective method which was based on alkaline hydrolysis and absorption on Whatman 42 filter-paper. The hydrolysis procedure was performed in methanol and sodium hydroxide solution at 100°C. The resulting solution was applied on Whatman 42 filter-paper and measured the fluorescence. The excitation wavelength and emission wavelength were at 365 nm and 460 nm respectively. The application of this method in the analysis of mebendazole in the pharmaceutical

formulations showed a good result with the coefficient of variation from 2.27 to 2.97 percent.

3. Chromatographic Methods

3.1 Thin-Layer Chromatographic Method (TLC)

The advantage of TLC is to separate mebendazole from other substances which interfere the absorption of UV-light. A.A. Patel et al. (19) showed that the assay of mebendazole by this method was possible. The mixture of formic acid, methanol and chloroform was used to dissolve mebendazole and the obtaining solution was applied as a strip on silica gel HF 254 plate. The separation was done by using a mixture of chloroform, ethyl acetate, acetone and formic acid as the developing solvent. An equal located band was extracted with chloroform to obtain a clear solution in which the absorbance was measured at 313 ± 2 nm against blank. The amount of mebendazole was calculated on the basis of the calibration of standard preparation which was prepared in the same manner.

3.2 High Pressure Liquid Chromatographic Methods (HPLC)

Many methods for the determination of mebendazole by HPLC have been reported by D.P. Wang (24), D. Mourot (25), G. Karlaganis (26), K.B. Alton (27) and R.J. Allon (28). Column, mobile phase, wavelength of UV-detector and conditions used were different in order to be applicable for each method. For example, D.P. Wang (24) assayed mebendazole in tablets by using μ Bondapak C 18 column, a 254 nm UV-detector, tetrahydrofuran : 0.5% formic acid (30:60) as mobile phase, and salicylamide as internal standard.

As has known that HPLC is the most valuable method in the determination of the substance in a small amount, especially in the biological fluid therefore, the determination of mebendazole in human plasma has been widely used at present (26-28).

4. Electrometric Methods

4.1 D.C. Polarographic Method

Determination of mebendazole by polarography has been reported by S. Pinzauti (29) and A.S. Boneva (30). S. Pinzauti (29) determined mebendazole in tablets by dissolving in perchloric acid. The polarographic wave probably was based on the reduction of the benzoyl group. The polarogram recorded from -0.6 V at 25°C with a dropping-mercury electrode, a platinum-wire auxillary electrode, and a silver-silver chloride reference electrode, gave a good result in the analysis of mebendazole. The optimum concentration which could be quantitated was between 5 and 50 mcg/ml of mebendazole.

4.2 Potentiometric Method

Potentiometric method for the determination of mebendazole in raw material was recommended by the USP XXI (18). Mebendazole was dissolved in glacial acetic acid and titrated with perchloric acid, using calomel-glass electrode.

III Statements of Problem

Mebendazole, the anthelmintic drug, is an aromatic ketone, then it can react with 2,4-DNPH to yield an orange precipitate of 2,4-DNPH' zone derivative which shows maximum absorption in chloroform-methanol (2:1) mixture at 393 ± 2 nm. Before measuring the absorption,

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the precipitate should be filtered and washed to remove the excess reagent which interferes the absorption. From this advantage, the determination of mebendazole in tablet formulation can be possible. Various conditions affecting the reaction were examined to choose the optimum conditions for the determination of mebendazole in tablet. The results obtained were also compared to those obtained from the USP XXI method.

The outline of this thesis is based on the following statementes :

1. The reaction of mebendazole and 2,4-DNPH is studied. The resulting 2,4-DNPH' zone derivative is examined and identified by thin-layer chromatography, infrared spectrometry, ultraviolet-visible spectrometry, nuclear magnetic resonance spectrometry and mass spectrometry.

2. Various conditions affecting the reaction are examined : acidity, temperature, time, mole ratio of sample to reagent, maximum absorption wavelength, and linearity of absorbance-concentration relationship. The optimum conditions for the assay are selected.

3. The accuracy and the precision of the 2,4-dinitrophenydrazine method are measured by determining the percent recovery and the percent coefficient of variation which are compared to those obtained from the official USP XXI method.

4. The 2,4-dinitrophenylhydrazine method is applied to determine the content of mebendazole in tablet formulation of five commercial products. The results obtained are compared to those obtained from the official USP XXI method.