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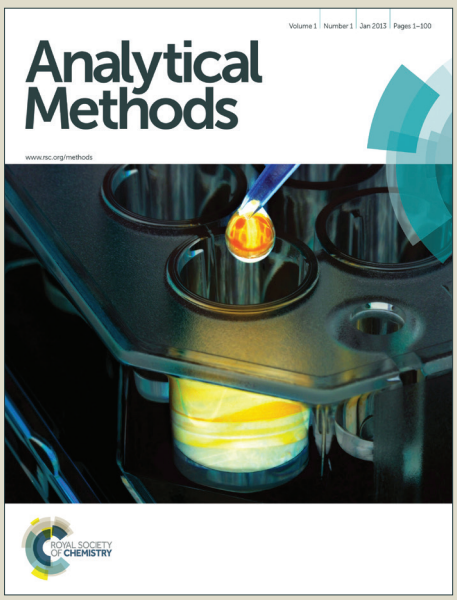

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A Simple Spectrophotometric Method for the Determination of Trace Level of Molybdenum in Real, Environmental, Biological and Soil Samples Using Benzoylacetone-benzoylhydrazone

M. Jamaluddin Ahmed*, M. Nasir Uddin, Tasnim Zannat and Salma Sultana

Laboratory of Analytical Chemistry, Department of Chemistry, University of Chittagong, Chittagong-4331, Bangladesh, E-mail: pmjahmed55@gmail.com

Abstract

A very simple, ultra-sensitive and highly selective non-extractive spectrophotometric method for the determination of trace amount of molybdenum(VI) in solution using benzoylacetone-benzoylhydrazone (Bzac-BH) has been developed. Bzac-BH reacts in a slightly acidic (0.0003-0.002M H_2SO_4) with molybdenum (VI) to give an orange chelate which has an absorption maximum at 447nm. The reaction is instantaneous and the absorbance remains stable for over 12h. The average molar absorption co-efficient and Sandell's sensitivity were found to be 6.3×10^5L $mol^{-1}cm^{-1}$ and 15ng cm^{-2} of molybdenum (VI), respectively. Linear calibration graphs were obtained for 0.05 - 2mgL^{-1} of molybdenum(VI). The stoichiometric composition of the chelate is 1:1 (Mo:Bzac-BH). A large excess of over 50 cations, anions and complexing agents (like, chloride, phosphate, azide, thio-sulfate, thio-urea, SCN^- etc.) do not interfere in the determination. The developed method was successfully used in the determination of molybdenum in several Standard Reference Materials (alloys and steels) as well as in some environmental waters (inland and surface), biological samples (human blood and urine), soil samples, solution containing both molybdenum(V) and molybdenum(VI) and complex synthetic mixtures. The method has high precision and accuracy (s = ± 0.01 for 0.5mgL^{-1}).

Keywords: *Spectrophotometry; molybdenum determination; Bzac-BH; alloys, steels, Environmental; biological; soil samples.*

***Corresponding author**

Introduction

Molybdenum is an essential trace element in animal physiology[1] as a cofactor for the enzymes xanthines oxidase and aldehyde oxidase. In plants and animals, molybdenum enzymes catalyze the oxidation and sometimes reduction of certain small molecules, as a part of the regulation of nitrogen, sulfur and carbon cycles[2]. Protein synthesis, metabolism and growth[3] in living organisms are affected by molybdenum concentrations. The human body contains about 0.07mg of molybdenum per kilogram of weight[1] in the lung, kidneys, liver[4] and it is also present within human tooth enamel to prevent its decay[3]. The average daily intake of molybdenum varies between 0.12mg and 0.24mg, but it depends on the molybdenum content of the food[3]. However, an extremely high concentration of molybdenum reverses the trend and can act as an inhibitor in both purine metabolism and other processes. Chronic exposure to excess molybdenum in human is characterized by high uric acid levels in serum and urine, loss of appetite, diarrhea, anemia and slow growth. Severe gastrointestinal irritation with diarrhea, coma and death from cardiac failure are also the symptoms of acute Mo toxicity. Understanding the behavior of molybdenum in the biological and environmental system is, therefore, of major concern[4]**.** That is why the determination of molybdenum(VI) in environment is of great concern. The concentration of molybdenum can be found very low in natural matrices. Therefore, it is important from an analytical point of view to find sensitive methods for its determination. This can be carried out by atomic absorption spectrophotometry at the 313.3nm Mo resonance line, using either flame (nitrogen oxide-acetylene)[5] or electrothermal atomization[6] as well as by plasma emission[7]. Both methods are disadvantageous in terms of cost and the instruments used in routine analysis. AAS often lacks sensitivity, and is affected by the matrix conditions of samples, such as salinity[8]. Catalytic solvent extractive methods are highly sensitive and less expensive, but generally lack simplicity[9].

The aim of this study was to develop a simpler direct spectrophotometric method for the trace determination of molybdenum. In the search for a more sensitive reagent, in this work a new reagent benzoylacetone-benzoylhydrazone(Bzac-BH) was synthesized according to the method of Sacconi[10] and a color reaction of Bzac-BH with Mo(VI). Bzac-BH has not previously been used for the spectrophotometric determination of any metal. This paper reports first time on its use in a very sensitive, highly specific spectrophotometric method for the trace determination of

molybdenum. The method possesses distinct advantages over existing methods[11-25] (**Table 1**) with respect to sensitivity, selectivity, range of determination, simplicity, speed, pH/acidity range, thermal stability, accuracy, precision and ease of operation. The method is based on the reaction of non-absorbent Bzac-BH in a slightly acidic solution (0.0003-0.002M H_2SO_4) with Mo (VI) to produce a highly absorbent orange colored chelate product, followed by a direct measurement of the absorbance in an aqueous solution. With suitable masking, the reaction can be made to be highly selective and the reagent blank solution does not show any absorbance.

Experimental Section

Apparatus

A Shimadzu (Kyoto, Japan) (Model-1800) double-beam UV/VIS spectrophotometer and a Jenway (England, UK) (Model-3010) pH meter with combination of electrodes were used for measurements of the absorbance and pH, respectively. A Buchi melting point apparatus (Model-M-365) and Eltra elemental analyzer were used to measure the melting point and thermogravimetric measurements of the reagent. A Shimadzu (Model-AA7000) atomic absorption spectrometer equipped with a microcomputer-controlled nitrous oxide-acetylene flame was used to compare the results. Infrared spectrum was recorded with FTIR Spectrophotometer, Shimadzu (Model-IR Prestige 21, Detector-DTGS KBr) in the range 7500-350 cm^{-1}.

Synthesis of the reagent

The reagent was synthesized in the laboratory according to the method recommended by Sacconi[10] and Salam[26]**.** The reagent benzoylacetone-benzoylhydrazone (Bzac-BH) was synthesized by two steps which is shown in reaction scheme1.

Characterization of the reagent

The reagent was characterized by taking melting point, elemental analysis, FTIR spectrum and thermogravimetric analysis. The melting point of the reagent was 135-137ºC (Lit. 135ºC)[26].

The results of elemental analysis (C= 42.01 %, N=5.77 %, H=2.96 %) of the reagent was in good coincidence with the calculated values (C=42.06 %, N=5.77 %, H=2.89%). The FTIR spectrum of prepared reagent (Bzac-BH) shown in **Fig. 1.** The presence of peak at 1581.63cm^{-1} in **Fig. 1**

was due to the characteristics C=N double bond peak ($\nu^{C=N}$, 1580-1660cm^{-1})[26], the peak at 1660.71cm^{-1} in **Fig. 1** was due to the characteristics C=O double bond peak ($\nu^{C=O}$, 1600-1735cm^{-1}), the presence of peak at 3230.77cm^{-1} in **Fig. 1** was due to the characteristics N-N single bond peak (ν^{N-N}, 3000-3500cm^{-1}), the presence of peak at 1600.92cm^{-1} in **Fig. 1** was due to the characteristics C=C double bond peak ($\nu^{C=C}$, 1581-1620cm^{-1})[26] of the Bzac-BH.

Both FTIR spectrum and elemental analysis data indicated the formation of the reagent Bzac-BH. The steadiness of the thermogravimetric curve obtained for about 1g of the reagent at (80-90) ºC, indicating that the reagent did not contain any moisture which is shown in **Fig.2.**

Reagents and solutions

All chemicals used were of analytical-reagent grade of the highest purity available. Doubly distilled de-ionized water and HPLC-grade absolute ethanol, which is non-absorbent under ultraviolet radiation, were used throughout the experimental works. Other necessary solutions were prepared according to our previous paper[27-39]. Glass vessels were cleaned by soaking in an acidified solutions of $KMnO_4$ or $K_2Cr_2O_7$, followed by washing with nitric acid (1+1) and were rinsed several times with high-purity de-ionized water. Stock solutions and environmental water samples (1000mL each) were kept in polypropylene bottles containing 1mL of concentrated HNO_3. More rigorous contamination control was used when the molybdenum levels in the specimens were low.

Bzac-BH stock solution, 3.8 × 10^{-4}M

The reagent stock solution was prepared by dissolving the requisite amount of Bzac-BH, in a known volume of distilled ethanol. More dilute solutions of the reagent were prepared as required.

Mo(VI) stock solution (1.49 × 10^{-3}M)

A 100mL amount of stock solution (1 mg mL^{-1}) of hexavalent molybdenum was prepared by dissolving 184.0mg as Mo(VI) of purified-grade (E Merck proanalysis grade) ammonium molybdate tetrahydrate $(NH_4)_6Mo_7O_{24}.4H_2O$ (super special grade J. T. Baker) in doubly distilled de-ionized water and subsequently standardized gravimetrically by the 8-quinolinol[40]. More dilute standard solutions were prepared by appropriate dilution of aliquots from the stock solution with de-ionized water as and when required.

Molybdenum (V) stock solution

A 100ml amount of stock solution (1mg mL^{-1}) of pentavalent molybdenum was prepared by dissolving 284.7mg as Mo(V) of molybdenum (V) chloride (Aldrich A.C.S. grade) in doubly distilled deionized water containing 1-2ml of nitric acid (1+1). More dilute standard solutions were prepared by appropriate dilution of aliquots from the stock solution with deionozed water as and when required.

Potassium permanganate solution

A 1% potassium permanganate (Merck) solution was prepared by dissolving in de-ionized water. Aliquots of this solution were standardized with oxalic acid. Sodium azide solution (2.5 % w/v) (Fluka purity > 99%) was also used.

Tartrate solution

A 100mL stock solution of tartrate (0.01 % w/v) was prepared by dissolving 10mg of A.C.S.-grade (99%) potassium sodium tartrate tetrahydrate in (100mL) de-ionized water.

Aqueous ammonia solution

A 100mL solution of an aqueous ammonia solution was prepared by diluting 10mL concentrated NH_4OH (28-30%, A.C.S.-grade) to 100mL with de-ionized water. The solution was stored in a polypropylene bottle.

Other Solutions

Solutions of a large number of inorganic ions and complex agents were prepared from their Analar grade or equivalent grade water-soluble salts (or the oxides and carbonates in hydrochloric acid); those of niobium, tantalum, titanium, zirconium and hafnium were specially prepared from their corresponding oxides (Specpure, Johnson Matthey) according to the recommended procedures of Mukharjee[41]. In the case of insoluble substances, special dissolution methods were adopted[42].

Procedure

To 0.1-1.0mL of a neutral aqueous (pH 6) solution containing 0.5-20µg of molybdenum (VI) in a 10-mL calibrated flask was mixed with a 1:100-1:500 fold molar excess of the Bzac-BH reagent

solution (preferably 1mL of 3.8×10^{-4}M) followed by the addition of 0.3-2.0mL (preferably 1mL) of 0.01M of sulfuric acid. After 1 min, 5ml of ethanol was added and the mixture was diluted to the mark with de-ionized water. The absorbance was measured at 447nm against a corresponding reagent blank. The molybdenum content in an unknown sample was determined using a concurrently prepared calibration graph.

Results and Discussion

Factors Affecting the Absorbance

Absorption spectra

The absorption spectra of the Mo(VI)-Bzac BH system in 0.01M sulfuric acid medium was recorded using a spectrophotometer. The absorption spectra of the Mo(VI)-Bzac- BH is a symmetric curve with maximum absorbance at 447nm ; an average molar absorption coefficient of 6.3×10^{5}Lmol^{-1}cm^{-1} is shown in **Fig. 3.** Bzac-BH did not show any absorbance. In all instances, measurements were made at 447nm against a reagent blank.

Effect of solvent

As Bzac-BH is insoluble in water, an organic solvent was used for the system. Of the various solvents (benzene, chloroform, acetone, carbon tetrachloride, nitrobenzene, isobutyl alcohol, 1-butanol, isobutyl methyl ketone, ethanol and 1, 4-dioxane) studied, ethanol was found to be the best solvent for the system. No absorbance was observed in the organic phase with the exception of 1-butanol. In 50±2% (v/v) ethanolic medium however the maximum absorbance was observed; hence, a 50% ethanolic solution was used in the determination procedure (**Fig. 4**).

Effect of acidity

Of the various acids (nitric, sulfuric, hydrochloric and phosphoric) studied, sulfuric acid was found to be the best acid for the system. The absorbance was at a maximum and constant when the 10mL of solution (1mgL^{-1}) contained 0.3-2.0mL of 0.01M sulfuric acid at room temperature (25 ± 5°C). Outside this range of acidity, the absorbance decreased (**Fig . 5**). For all subsequent measurements 1mL of 0.01M sulfuric acid was added.

Effect of temperature

The molybdenum (VI)- Bzac-BH system attained maximum and constant absorbance at (15-40)°C temperature. For all subsequent measurements were done at room temperature (25 ± 5°C).

Effect of time

The reaction is instantaneous. The molybdenum (VI)-Bzac-BH system attained maximum and constant absorbance immediately (within 1min) after dilution the solution to the final volume, which then remained strictly unaltered for 12h .

Effect of reagent concentration

Different molar excesses of Bzac-BH were added to a fixed metal ion concentration and absorbances were measured according to the standard procedure. It was observed that at 1mg L^{-1} Mo(VI) metal, the reagent molar ratios of 1:100-1:500 produced a constant absorbance of the Mo-chelate (**Fig . 6**). Greater excesses of reagent were not studied. For all subsequent measurements, 1mL of 3.8×10^{-4}M Bzac-BH reagent was added.

Calibration Graph (Beer's Law and Sensitivity)

The well-known equation for spectrophotometric analysis in very dilute solutions derived from Beer's law. The effect of metal concentration was studied over 0.01-100mgL^{-1} distributed in four different sets(0.01-0.1,0.1-1, 1-10 and 10-100mgL^{-1}) for the convenience of measurement. The absorbance was linear for 0.05-2mgL^{-1} of molybdenum at 447nm representing three graphs (0.05-0.1, 0.1-1 and 0.5-2mgL^{-1}). Of the three calibration graphs, one showed the limit of the linearity range (**Fig.7**); the next two were straight-line graphs passing through the origin (R^2 = 0.9997). The molar absorption coefficient and Sandell's sensitivity[43] were found to be 6.3×10^5L$mol^{-1}cm^{-1}$ and 15ng cm^{-2} of molybdenum(VI), respectively. The selected analytical parameters obtained with the optimization experiments are summarized in **Table 2**.

Effect of Foreign Ions

The effect of over 50 ions and complexing agents on the determination of only 1mgL^{-1} of molybdenum (VI) was studied. The criterion for an interference[44] was an absorbance value varying by more than ±5% from the expected value for molybdenum alone. The results are summarized in **Table 3**. As can be seen, a large number of ions have no significant effect on the

determination of molybdenum. The most serious interference were from Fe(II) and Fe(III)ions. Interference from these ions are probably due to complex formation with Bzac-BH.

The greater tolerance limits for these ions can be achieved by using several masking methods. In order to eliminate the interference of Fe(II) and Fe(III)ions, thio-urea and thiocyanate can be used as masking agents. A 10-fold excess of Fe(II) and Fe(III) ions could be masked with thiourea and thiocyanate. During the interference studies, if a precipitate was formed, it was removed by centrifugation. Fe(II) and Fe(III) ions interference when present in amounts of excess of molybdenum(VI). Interference from these three metal ions have been effectively removed by a short single-step ion-exchange separation process, using an Amberlite XAD-8 resin (100-200 mesh) anion exchanger[45].

Precision and Accuracy

The precision of the present method was evaluated by determining different concentrations of molybdenum (each analyzed at least five times). The relative standard deviation (n=5) was 0-2% for 0.5-20μg of molybdenum (VI) in 10-mL, indicating that this method is highly precise and reproducible (**Table 3**). The detection limit (3s of the blank) and Sandell's sensitivity (concentration for 0.001 absorbance unit) for molybdenum (VI) were found to be 0.3μg L^{-1} and 15ng cm^{-2}, respectively. The results for total molybdenum were in good agreement with certified values (**Table 4**). The reliability of our molybdenum-chelate procedure was tested by recovery studies. The average percentage recovery obtained for addition of molybdenum (VI) spike to some environmental water samples was quantitative, as shown in **Table 5.** The method was also tested by analyzing several synthetic mixtures containing molybdenum (VI) and diverse ions (**Table 6**). The results of biological analyses by the spectrophotometric method were in excellent agreement with those obtained by AAS (**Table 7**). The results of speciation of molybdenum (V) and molybdenum (VI) in mixtures were highly reproducible (**Table 9**). Hence, the precision and accuracy of the method were found to be excellent.

Composition of the Absorbent complex

Molar ratio[46] method and the Job's method[47] of continuous variation were applied to ascertain the stoichiometric composition of the complex. A molybdenum-Bzac-BH (1:1) complex was indicated by both methods.

Applications

The present method was successfully applied to the determination of molybdenum (VI) in a series of synthetic mixtures of various compositions (**Table 4**) and also in a number of real samples e.g. several Certified Reference Materials (CRM) (**Table 5**). The method was also extended to the determination of molybdenum in a number of environmental, biological, soil samples. In view of the unknown composition of environmental water samples, the same equivalent portions of each such samples were analyzed for molybdenum content; the recoveries in both the "spiked" (added to the samples before the mineralization or dissolution) and the "unspiked" samples are in good agreement (**Table 6**). The results of biological analyses by spectrophotometric method were found to be in excellent agreement with those obtained by AAS (**Table 7**). The results of soil sample analysis by the spectrophotometric method are shown in **Table 8**. The speciation of molybdenum (V) and molybdenum (VI) are shown in **Table 9**. The precision and accuracy of the method were excellent.

Determination of Molybdenum in Synthetic Mixtures

Several synthetic mixtures of varying compositions containing molybdenum (VI) and diverse ions of known concentrations were determined by the present method and the results were found to be highly reproducible as shown in **Table 4**. Accurate recoveries were achieved in all solutions.

Determination of Molybdenum in Alloys and Steels (Certified Reference Materials)

A 0.1-g amount of an alloy or steel sample containing 0.15 - 4.95% of molybdenum was weighed accurately and placed in a 50mL Erlenmeyer flask following a method recommended by Parker[48]. To it, 10mL of 20% sulfuric acid was added while carefully covering with a watch glass until the brisk reaction subsided. The solution was heated and simmered gently after the addition of 5mL of concentrated HNO_3 until all carbides were decomposed. Then, 2mL of 1:1 (v/v) H_2SO_4 was added and the solution was carefully evaporated to dense white fumes to drive off the oxides of nitrogen, and then cooled to room temperature (25 ± 5)°C. After suitable dilution with de-ionized water, the contents of the Erlenmeyer flask were warmed so as to dissolve the soluble salts. The solution was then cooled and neutralized with a dilute NH_4OH in the presence of 1-2ml of 0.01%(w/v) tartrate solution. The resulting solution was filtered, if

necessary, through a Whatman No. 40 filter paper into a 25mL calibrated flask. The residue (silica and tungstenic acid) was washed with a small volume of hot (1 + 99) H_2SO_4, followed by water; the volume was made up to the mark with de-ionized water.

A suitable aliquot (1-2mL) of the above-mentioned solution was taken into a 10-mL calibrated flask and the molybdenum(VI) content was determined; as described under Procedure using thio-urea or thiocyanate as masking agent. Based on five replicate analyses, average molybdenum concentration determined by spectrophotometric method was in close agreement with the certified values (**Table 5**).

Determination of Molybdenum in Environmental Water Samples

Each filtered (with Whatman No. 40) environmental water sample (1000mL) was evaporated nearly to dryness with a mixture of 5mL concentrated H_2SO_4 and 10mL of concentrated HNO_3 in a fume cupboard, following a method recommended by Greenberg *et al.*[49] and was then cooled to room temperature. The residue was then heated with 10mL of de-ionized water in order to dissolve the salts. The solution was then cooled and neutralized with dilute NH_4OH solution. The resulting solution was then filtered and quantitatively transferred into a 25mL calibrated flask and made up to the mark with de-ionized water.

An aliquot (1mL) of this preconcentrated water sample was pipetted into a 10mL calibrated flask and the molybdenum content was determined as described under the Procedure using thiourea or thiocyanate as masking agent. The analysis of environmental water samples from various sources for molybdenum is shown in **Table 6**.

Most spectrophotometric methods for determination of molybdenum in natural and sea water require preconcentration of molybdenum[48]. The concentration of molybdenum in natural water and sea water is a few ng mL^{-1} in Taiwan[49]. The mean concentration of molybdenum found in U.S. drinking water is <10ng mL^{-1} [49].

Determination of Molybdenum in Biological Samples

Human serum (5-10mL) or urine (10-20mL) was taken into a 100mL micro-Kjeldahl flask. A glass bead and 10mL of concentrated nitric acid were added, and the flask was placed on the digester under gentle heating. When the initial brisk reaction was completed, the solution was removed and cooled following a method recommended by Stahr[50]. A 1mL volume of

concentrated sulfuric acid was carefully added, followed by the addition of 1mL of 70% perchloric acid; and heating was continued to dense white fumes, while repeating nitric acid addition if necessary. Heating was continued for at least 0.5h and then cooling was applied. The content of the flask was filtered and neutralized with dilute NH_4OH solution. The resultant solution was then filtered and transferred quantitatively into a 10-mL calibrated flask and made up to the mark with de-ionized water.

A suitable aliquot (1mL) of the final solution was pipetted out into a 10-mL calibrated flask and the molybdenum content was determined as described under procedure using thiourea or thiocyanate as masking agent. The results of biological analyses by the spectrophotometric method were found to be in excellent agreement with those obtained by AAS. The results are shown in **Table 7**.

The abnormally high value for the gastrointestinal disturbance and cardiovascular patients is probably due to the involvement of high molybdenum concentrations with Zn and As. The occurrence of such high molybdenum contents is also reported in gastrointestinal disturbance and cardiovascular patients from some developed countries[12].

Determination of Molybdenum in Soil Samples

An air-dried homogenized soil sample (100g) was accurately weighed and placed in a 100mL micro-Kjeldahl flask. The sample was digested in the presence of an oxidizing agent, following a method recommended by Jackson[51]. The content of the flask was filtered through a Whatman No.40 filter paper into a 25mL calibrated flask and neutralized with dilute NH_4OH solution. The resulting solution was then filtered and quantitatively transferred into a 25mL calibrated flask and made up to the mark with de-ionized water.

A suitable aliquot (1mL) of the final solution was pipetted out into a 10mL calibrated flask and the molybdenum content was determined as described under procedure using thio-urea or thiocyanate as masking agent. The molybdenum content was then determined by the above procedure and quantified from a calibration graph prepared concurrently. The results are shown in **Table 8**.

Determination of Molybdenum(V) and Molybdenum(VI) speciation in Mixtures

Suitable aliquots (1-2mL) of molybdenum (V + VI) mixtures (preferably 1:0.1, 1:0.5, 1:1) were taken in a 25mL conical flask. A few drops of 0.01M sulfuric acid and 1-3mL of 1% (w/v) potassium permanganate solution were added to oxidize the pentavalent molybdenum. A 5mL volume of water was added to the mixtures, which were then heated on a steam bath for 10-15min, with occasional gentle shaking, and then cooled to room temperature. Then, 3-4 drops of a freshly prepared sodium azide solution (2.5% w/v) was added and heated gently with the further addition of 2-3mL of water, if necessary, for 5min to drive off the azide cooled to room temperature. The reaction mixtures was neutralized with dilute NH_4OH and transferred quantitatively into a 10-mL volumetric flask. 1ml of 3.8 x 10^{-4}M Bzac-BH reagent solution was added, followed by the addition of 1mL of 0.01M H_2SO_4.It was made up to the mark with de-ionized water. The absorbance was measured after 1 min at 447nm against a reagent blank. The total molybdenum content was calculated with the help of a calibration graph.

An equal aliquot (1-mL) of the above molybdenum (V + VI) mixture was taken into a 25mL beaker. 1mL of 0.01%(w/v) tatrate was added to mask molybdenum (V) and neutralize with dilute NH_4OH. After, the content of the beaker was transferred into a 10mL volumetric flask, 1ml of 3.8 x 10^{-4}M Bzac-BH reagent solution was added, followed by the addition of 1mL of 0.01M H_2SO_4. It was made up to the mark with de-ionized water. After 1min the absorbance was measured at 447nm against a reagent blank, as before. The molybdenum concentration was calculated in mgL^{-1} or μgL^{-1} with the aid of a calibration graph. This gives a measure of molybdenum (VI) originally present in the mixture.This value was substracted from that of the total molybdenum to determine the molybdenum (V) present in the mixture. The results were found to be highly reproducible. The occurrence of such reproducible results is also reported for different oxidation states of molybdenum[48]. The results of a set of determination are given in **Table 9**.

Conclusions

A new, simple, sensitive, selective and inexpensive method with Mo VI -Bzac-BH complex was developed successfully for the determination of molybdenum in some industrial, environmental, biological and soil samples, for continuous monitoring to establish the trace levels of

molybdenum in different sample matrices. The similar methods[28-39] have also been reported previously. Although many sophisticated techniques, such as pulse polarography, HPLC, NAA, AAS, ICP-OES and ICP-MS, are available for the determination of molybdenum at trace levels in numerous complex materials, factors such as the low cost of the instrument, easy handling, portable, lack of any requirement for consumables, and almost no maintenances, have caused spectrophotometry to remain a popular technique, particularly in laboratories of developing countries with limited budgets. The sensitivity in terms of the molar absorptivity (ε = 6.3 x 10^5Lmol^{-1} cm^{-1}) and precision in terms of the relative standard deviation (0-2%) of the present method are very reliable for the determination of molybdenum in real samples down to (ng g^{-1}) levels in an aqueous medium at room temperature (25 ± 5^o C).

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Caption of Figures

Fig.1. FTIR spectrum of benzoylacetone-benzoylhydrazone, (Bzac-BH)

Fig.2. Thermogravimetric curve of benzoylacetone-benzoylhydrazone (Bzac-BH) at 80-90ºC

Fig.3. A and B absorption spectra of Mo^{VI}-Bzac-BH system and the reagent blank (λ_{max} = 447 nm) in aqueous solutions.

Fig.4. Effect of the solvent on the absorbance of the Mo^{VI} –Bzac-BH system

Fig.5. Effect of the acidity on the absorbance of the Mo^{VI} –Bzac-BH system

Fig.6. Effect of reagent [Mo^{VI} : Bzac-BH molar concentration ratio] on The absorbance of Mo^{VI}-Bzac-BH system.

Fig.7. Calibration graph : 0.5-2 mgL^{-1} of Mo(VI)

Fig.8. Composition of Mo^{VI}-BSOPD complex by the Mole ratio method in aqueous solution (1:1 complex formed).

Scheme 1: Synthesis of the reagent Benzoylacetone-benzoylhydrazone (Bzac-BH)

Table 1. Review of reagents for spectrophotometric determination of molybdenum(VI)

Reagent	λ_{max} /nm	Molar absorptivity € ($L\ mol^{-1}cm^{-1}$)	Beer's law range/$mg.L^{-1}$	Remarks	References
Amberlite XAD-8	462	-	-	i) Less sensitive. ii) Less selective due to much interference. iii) Molar absorptivity was not mentioned.	11
4,5 dibromo-2-nitrophenyl fluorane	540	9.5×10^4	0-14	i) Less selective due to much interference. ii) Limited application iii)Less sensitive.	12
Ammonium thiocyanate	470	1.63×10^4	0-60	i) Less sensitive. ii) Interference was many. iv)Limited application.	13
Salicylfluorine	523.0	1.25×10^5	0-0.56	i) pH- dependent ii) Less selective due to much interference. iii)Less sensitive. iv)Application was limited.	14
Methylene blue and hydrazine hydrochloride	-	3.2×10^4	0-1/25mL	i) pH- dependent ii) Limited applications iii)Less selective due to much interference. iv)Less sensitive.	15
Bromopyrogallol red (BPR)	-	1.3×10^4	0.06–0.8	i) Less sensitive. ii) Interference was many. iii) Less selective.	16
Silycylfluorane	522	1.50×10^4	-	i) Less sensitive. ii) Limited applications. iii) Less selective due to much interference	17
Morin	413	2.71×10^4	0.1-12	i) Less sensitive. ii) pH- dependent. iii) Less selective due to much Interference. iv) Limited application.	18
3,5-dibromo-4-hydroxyphenylflurone	470	3600±50	2-20	i) Less sensitive. ii) pH- dependent. iii) Less selective due to much interference iv) Limited application.	19
N1-hydroxy-n1-p-tolyl-n2-b-naphthylbenzamidine hydrochloride	530	1.35×10^5	0-6	i) Less sensitive. ii) Interference was many. iii) Less selective. iv) Limited application.	20
5.7dibromo - 8-hydroxyquinoline(DBHQ)	401	1.38×10^3	0.1-50	i)Limited applications ii)Less sensitive. iii)Less selective due to some interference.	21
p-carboxyphenylfluorone (p-CPF)	531	1.032×10^5	0-2	i) Less sensitive. ii) Interference was many. iii) Less selective due to much interference iii) Limited application.	22
Thiocyanate	461	5.6×10^3	-	i) Less sensitive. ii) Interference was many. iii) Limited application. iv) Less selective due to much interference	23
2- hydroxy-3-methoxy benzaldehyde thiosemicarbazone (HMBATSC	385	2.3×10^4	0.24-4.32	i) pH- dependent. ii) Interference was not studied. iii) Limited application.	24
Cinnamaldehyde-4-hydroxybenzoylhydrazone (CHBH)	404	6.82×10^4	0.047-5.0	i) Less sensitive. ii) pH- dependent. iii) Less selective due to much interference. iv) Limited application.	25
Benzoylacetone-benzoylhydrazone (Bzac-BH) **(Present method)**	447	6.3×10^5	0.06 - 2	i) Non-extractive. ii) Highly selective and Highly sensitive. iii) Aqueous reaction medium. iv) Color stable more than 12 h at 25 ± 5^0 C . v) Simple and rapid. vi) Carcinogenic solvents have been avoided.	**Present method**

Table 2 . Selected analytical parameters obtained with the optimization experiments.

Parameters	Studied range	Selected value
Wavelength / λ_{max} (nm)	200-800	447
Acidity / M H_2SO_4	0.0001-0.1	0.0003-0.002 (Preferably 0.001)
pH	3.0-1.0	2.52-1.70 (Preferably 2)
Time / h	0 - 72	1min-12 h (Preferably 1 min)
Temperature / °C	10-70	15-40 (Preferably 25 ± 5)
Reagent (fold molar excess, M:R)	1:1 - 1:500	1:100 - 1:500 (Preferably 1: 100)
Linear range/mg L^{-1}	0.01-100	0.05 - 2
Molar absorption coefficient / L mol^{-1} cm^{-1}	5.6 x 10^5 – 7.0 x 10^5	6.3 x 10^5
Sandell's sensitivity/ng cm^{-2}	1-100	15
Detection limit/ μg L^{-1}	0.01-10	0.3
Reproducibility (% RSD)	0 - 10	0 – 2
Regression Co-efficient (R^2)	0.9992-0.9999	0.9997

Table 3. Table of tolerance limits of foreign ions

Species x	Tolerance ratio x/Mo (w/w)	Species x	Tolerance ratio x/Mo (w/w)
Ammonium (I)	100	Lead (II)	10
Arsenic (III)	100	Magnesium	100
Arsenic (V)	100	Manganese(II)	10
Aluminium	100	Mercury (II)	100
Azide	100	Nitrate	100
Ascorbic acid	100	Nickel	100
Bromide	100	Potassium	20
Bismuth (III)	100	Phosphate	100
Barium	100	Selenium (VI)	50
Calcium	20	Selenium (IV)	50
Chloride	50	Silver	100
Cobalt (II)	100	Sodium	10
Cobalt (III)	100	Strontium	50
Chromium (III)	50	Sulfate	100
Chromium (VI)	100	Titanium(IV)	100
Cadmium	100	Tellurium(IV)	20
Carbonate	20	Thiocyanate	100
Copper (II)	100 [a]	Thiourea	100
Fluoride	10	Thiosulfate	100
Iodide	100	Tungsten (VI)	100
Iron (II)	10 [b]	Vanadium(V)	50[a]
Iron (III)	10 [b]	Zinc	100
Lithium	20		

Tolerance limit was defined as ratio that causes less than 5 percent interference

a. with 100 mg L^{-1} thiourea
b. with 50 mg L^{-1} thiocyanate

Table 4 . Determination of molybdenum in some synthetic mixtures

Sample	Composition of mixtures ($\mu g\ mL^{-1}$)	Molybdenum (VI) ($mg\ L^{-1}$)		
		Added	Found[a]	Recovery ± SD[b] (%)
A	Mo^{VI}	0.5 1.00	0.49 1.00	98 ± 0.4 100 ± 0.0
B	As in A + Al (25) + Ag(25)	0.5 1.00	0.505 1.02	101 ± 0.5 102 ± 0.6
C	As in B + Cd (25) + Ni^{2+}(25)	0.5 1.00	0.49 0.99	98 ± 0.5 99 ± 0.3
D	As in C + Mg (25) + Zn (25)	0.5 1.00	0.52 1.04	104 ± 1.2 104 ± 1.4
E	As in D + Ti^{IV} (25) + W^{VI} (25)	0.5 1.00	0.54 1.07	108 ± 1.5 107 ± 1.8

a. Average of five analyses of each sample
b. The measure of precision is the standard deviation

Table 5 . Determination of molybdenum in certified reference materials

Sample	Certified Reference Materials	Molybdenum (%)		
		In C.R.M sample	Found (n=5)	RSD[b]
1	BAS-CRM 64b high-speed steel (Cr, Mo, V and Te)	4.95	4.92 ± 0.04[a]	1.35
2	BAS-10g, high-speed brass (Cu, Fe, Pb, Ni, Sn, Al, Mo, Zn and Mn)	0.15	0.16 ± 0.08	2.5
3	GSBH 40101 - 96, $Cr_{12}MoV$-Dies steel (Cr, Ni, Mo, V, Cu, Co)	1.00	0.98 ± 0.05	1.8
4	YSBC1013-1-95.9$Cr_{17}MoVCo$ High tensil steel(C, Cr, Mo, V, Si, Mn and Co)	0.52	0.50 ± 0.06	2.0

a. The measure of precision is the standard deviation (s)
b. The measure of precision is the relative standard deviation(RSD)

Table 6. Determination of molybdenum in some environmental water samples

Sample		Molybdenum/μg L^{-1} Added	Molybdenum/μg L^{-1} Found [a]	Recovery ± s (%)	s_r [b] (%)
Tap water		0	6.5		
		100	105.0	99 ± 0.1	0.31
		500	507.0	100.1 ± 0.3	0.35
Well water		0	4.5		
		100	106.0	101.4 ± 0.4	0.29
		500	505.0	100.1 ± 0.3	0.16
River water	Karnaphully (upper)	0	15.0		
		100	115.0	100 ± 0.00	0.00
		500	516.0	100.2 ± 0.6	0.22
	Karnaphully (lower)	0	20.0		
		100	122.0	101.6 ± 0.6	0.25
		500	525.0	100.9 ± 1.0	0.23
	Halda (upper)	0	10.0		
		100	112.0	101.8±0.5	0.26
		500	510.0	100±0.00	0.00
	Halda (lower)	0	12.0		
		100	110.0	98±0.7	0.27
		500	512.0	100±0.0	0.00
Sea water	Bay of Bengal (upper)	0	13.0		
		100	111.0	98 ± 0.8	0.42
		500	515.0	101.7 ± 0.5	0.26
	Bay of Bengal (lower)	0	15.0		
		100	114.0	98 ± 0.7	0.15
		500	516.0	100.6 ± 0.8	0.20
Drain water	T. S. P. Complex [c]	0	75.0		
		100	180.0	102.8 ± 0.8	0.52
		500	570.0	99 ± 1.0	0.34
	PHP[d]	0	90.0		
		100	192.0	101 ± 1.2	0.26
		500	600.0	101.6 ± 1.5	0.18
	BSRM [e]	0	155.0		
		100	153.0	99 ± 1.1	0.46
		500	660.0	100.7 ± 1.2	0.35
	Elite Paint [f]	0	85.0		
		100	188.0	101.6 ± 1.6	0.29
		500	580.0	99 ± 1.8	0.47
	Eastern cables [g]	0	65.0		
		100	162.0	98 ± 1.3	0.55
		500	570.0	100.9 ± 1.5	0.37

a. Average of five replicate determinations of each Sample .
b. The measure of precision is the relative standard deviation(s_r)
c. T. S. P. complex Ltd., Patenga, Chittagong
d. PHP Steel Mill, Kumira, Chittagong
e. Bangladesh Steel and Roller Mill (BSRM) , Baizid Bosthami, Chittagong
f . Elite Paint, Nasirabad,Chittagong.
g. Estern Cables, Patenga, Chittagong

Table 7. Determination results of molybdenum for human fluids

Serial No.	Sample	Molybdenum / µg L^{-1}		Sample Source*
		AAS (n=5)	Proposed method (n = 5)	
1	Serum 1 Urine 1	133.0±1.0 37.7±1.2	131.5±1.2 35.4±1.0	Heart disease(Male)
2	Serum 2 Urine 2	168.0±1.3 42.0±1.5	165.6±1.5 39.5±1.3	Gastrointestinal disturbance patient(Male)
3	Serum 3 Urine 3	76.0±1.2 19.5±1.6	75.0±1.0 18.8±0.8	Hypertension Patient(Male)
4	Serum 4 Urine 4	16.5±0.8 5.0±0.6	15.5±0.7 4.5±0.5	Normal adult (Male)

*Samples were from Chittagong Medical College Hospital.

Table 8. Determination of molybdenum in some surface soil.

Serial No.	Molybdenum (mg kg^{-1}) [a]	RSD(%)	Sample Source
S_1 [b]	1.65	1.0	Agriculture soil (Chittagong University Campus)
S_2	1.05	0.8	Marin soil (Bay of Bengal)
S_3	1.34	1.2	Traffic soil (Kadamtali Bus Terminal)
S_4	1.56	1.3	Industrial soil (Estern Cables)
S_5	1.95	1.5	Industrial soil (T.S.P. Complex, Chittagong)
S_6	1.20	1.2	Road side soil (Dhaka-Chittagong Highway)
S_7	2.25	2.0	Paint soil (Elite Paint, Chittagong)

a. Average of five analyses of each sample
b. Composition of the soil samples: C, N, P, K, Na, Ca, Mg, Cu, Mo, Fe, Pb,V, Zn, Mn, Co, NO_3, SO_4 etc.

Table 9. Determination of molybdenum (V) and molybdenum (VI) in mixtures

Serial No.	Mo(VI) : Mo(V)	Mo, taken (mg L^{-1})		Mo, found (mg L^{-1})		Error (mg L^{-1})	
		Mo(VI)	Mo(V)	Mo(VI)	Mo(V)	Mo(VI)	Mo(V)
1	1:0.1	1.00	0.10	0.99	0.98	0.01	0.002
1	1:0.1	1.00	0.10	1.00	0.10	0.00	0.00
1	1:0.1	1.00	0.10	0.98	0.99	0.02	0.001
Mean error : Mo(VI) = ±0.01; Mo(V) = ± 0.001 Standard deviation : Mo(VI) = ± 0.005; Mo(V) = ± 0.0006							
1	1:0.5	1.00	0.50	0.99	0.498	0.01	0.002
1	1:0.5	1.00	0.50	0.98	0.499	0.02	0.001
1	1:0.5	1.00	0.50	0.99	0.498	0.01	0.002
Mean error : Mo(VI) = ± 0.013; Mo(V) = ± 0.0016 Standard deviation : Mo(VI) = ± 0.0058; Mo(V) = ± 0.0006							
1	1:1	1.00	1.00	0.98	0.99	0.02	0.01
1	1:1	1.00	1.00	1.00	0.98	0.00	0.02
1	1:1	1.00	1.00	0.99	0.98	0.01	0.02
Mean error : Mo(VI)= ± 0.01; Mo(V) = ± 0.01 Standard deviation : Mo(VI) = ± 0.005; Mo(V) = ± 0.006							

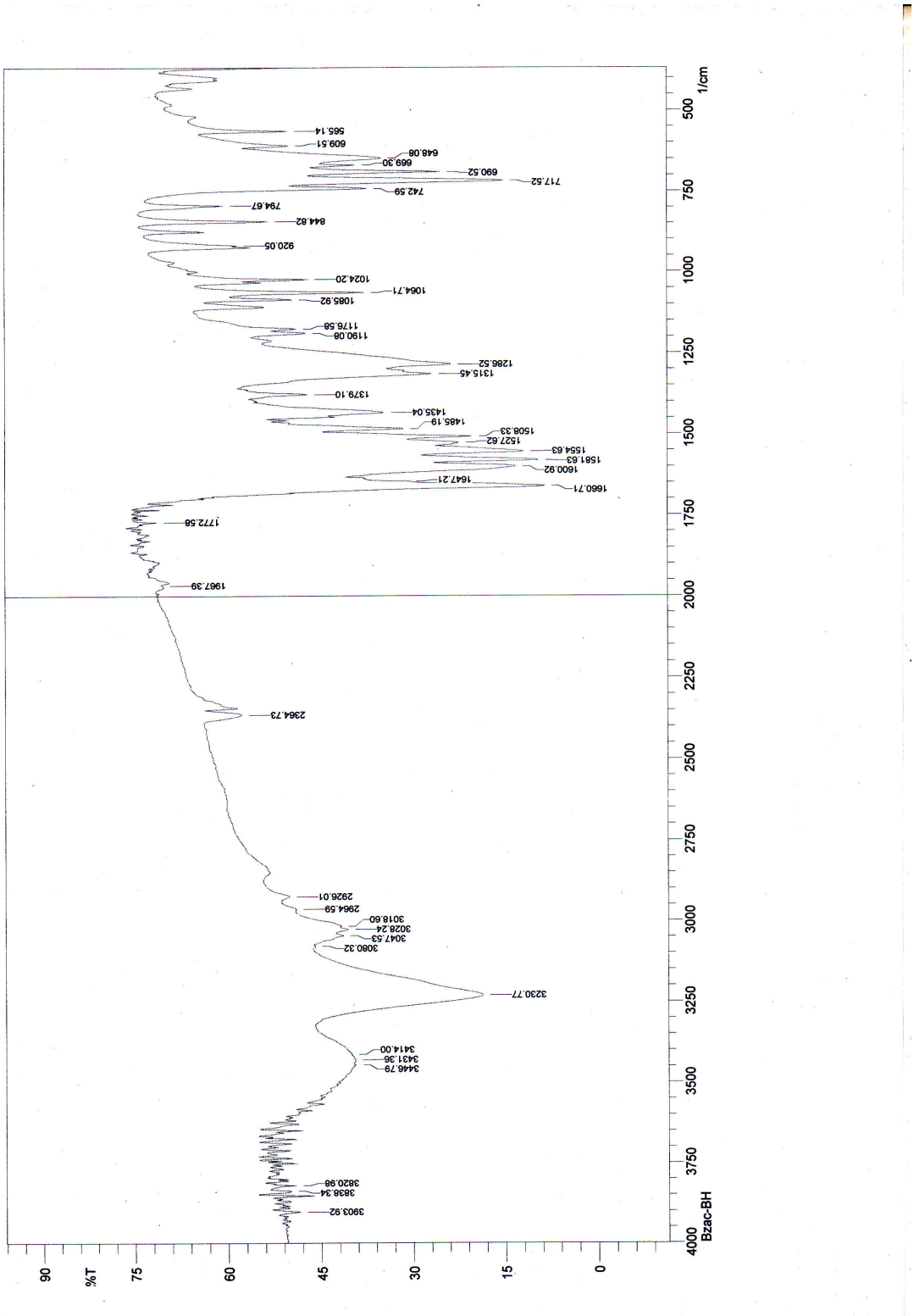


Fig. 1.

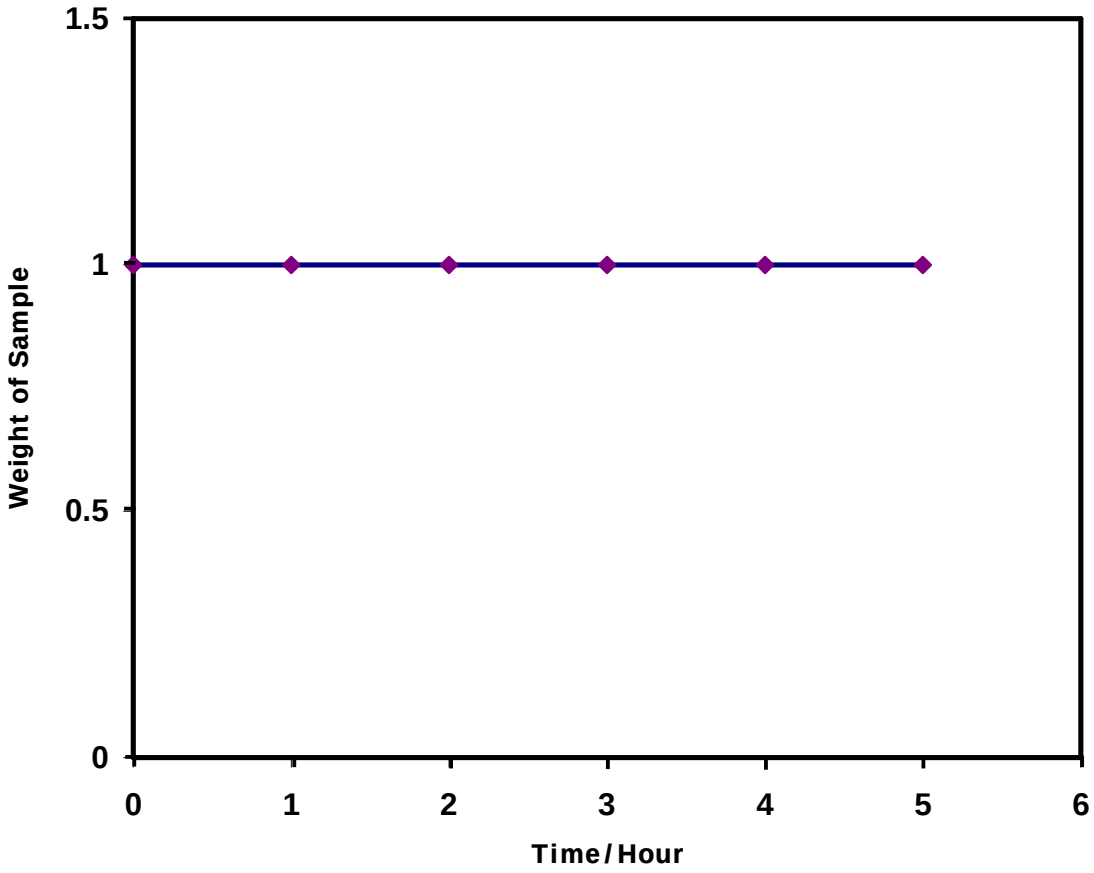


Fig . 2.

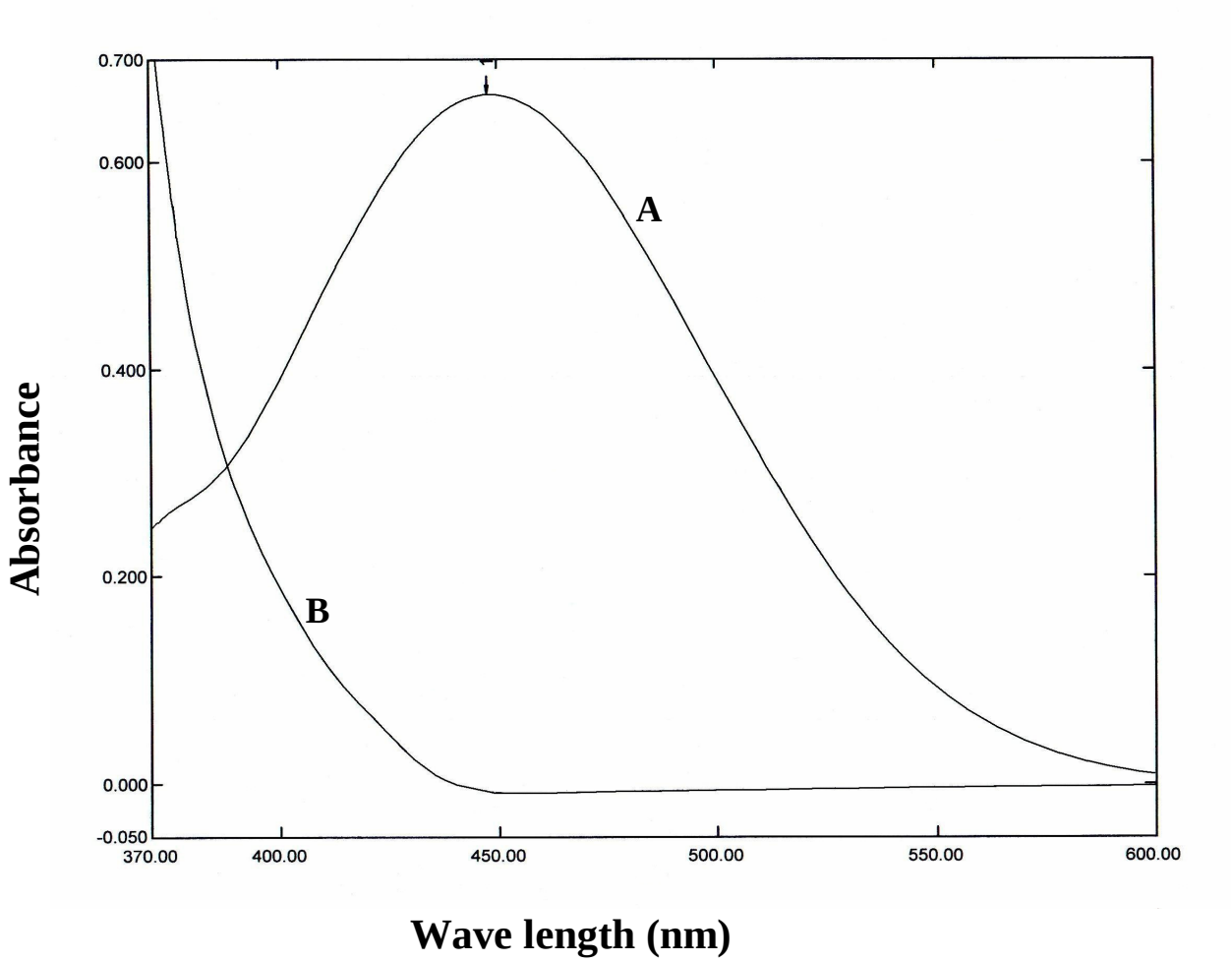


Fig. 3.

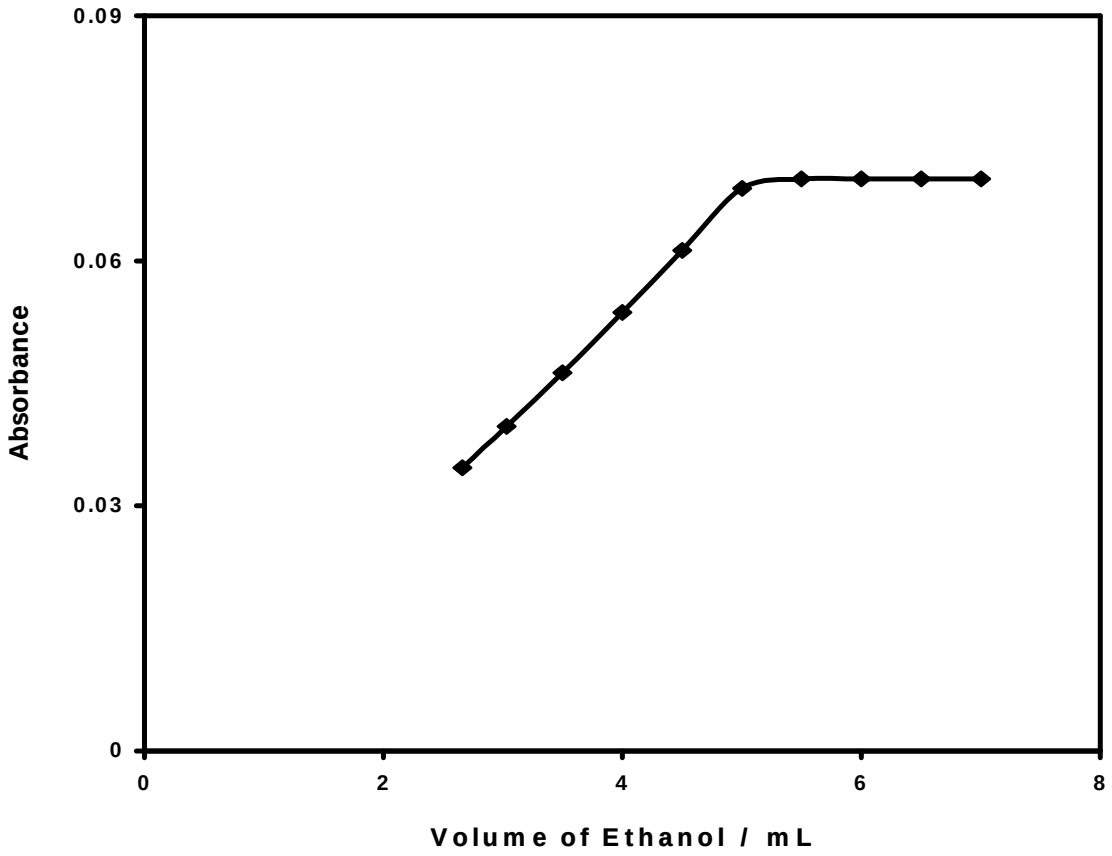


Fig . 4.

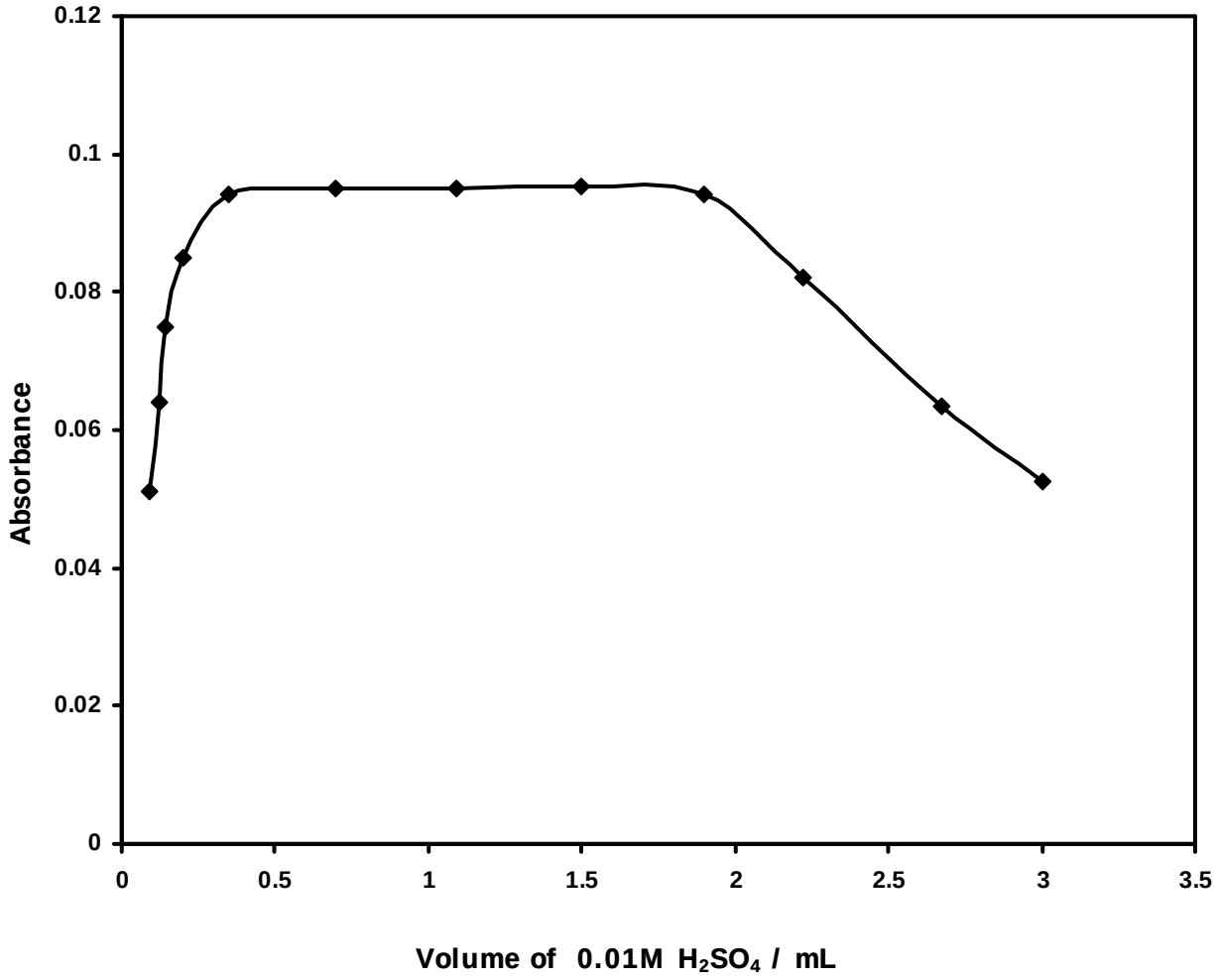


Fig. 5.

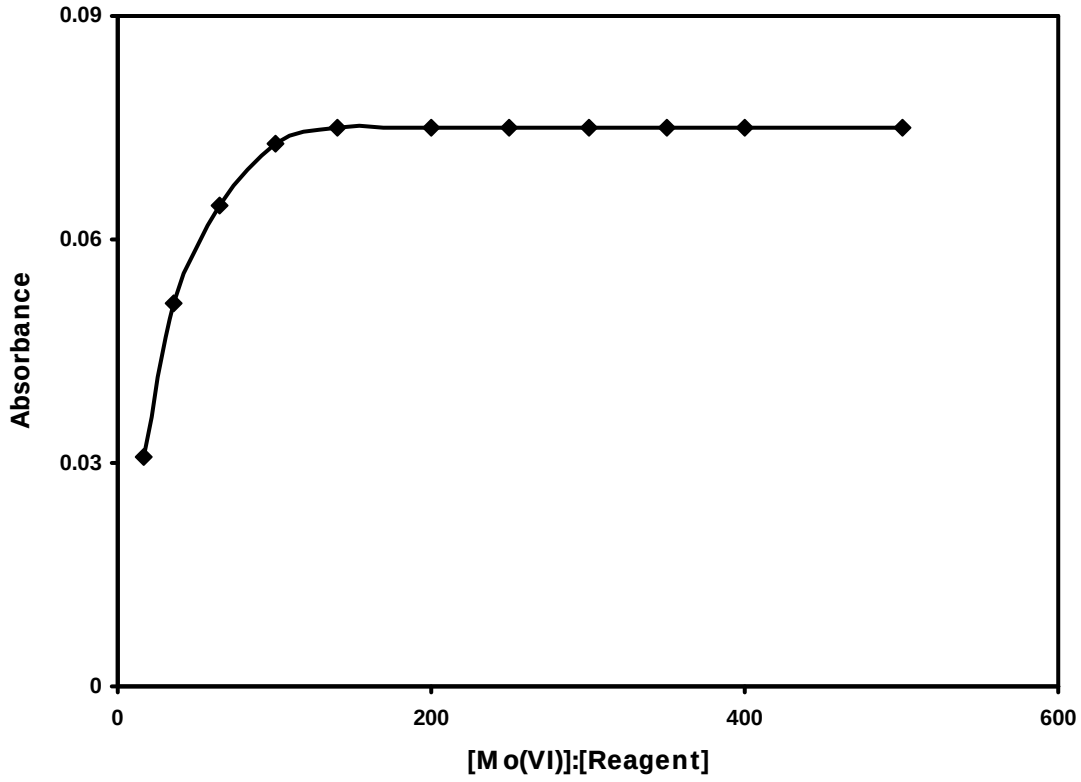


Fig. 6 .

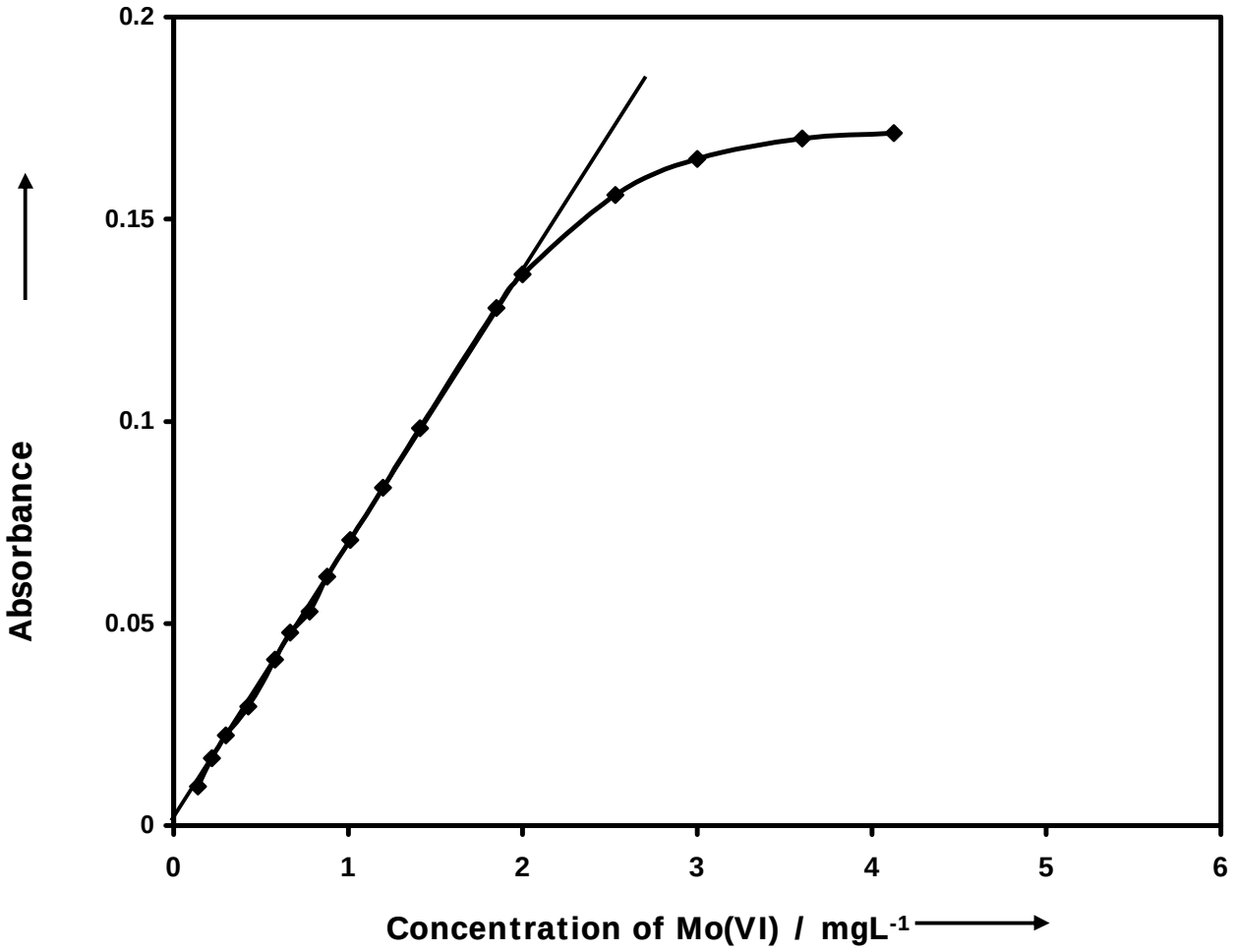
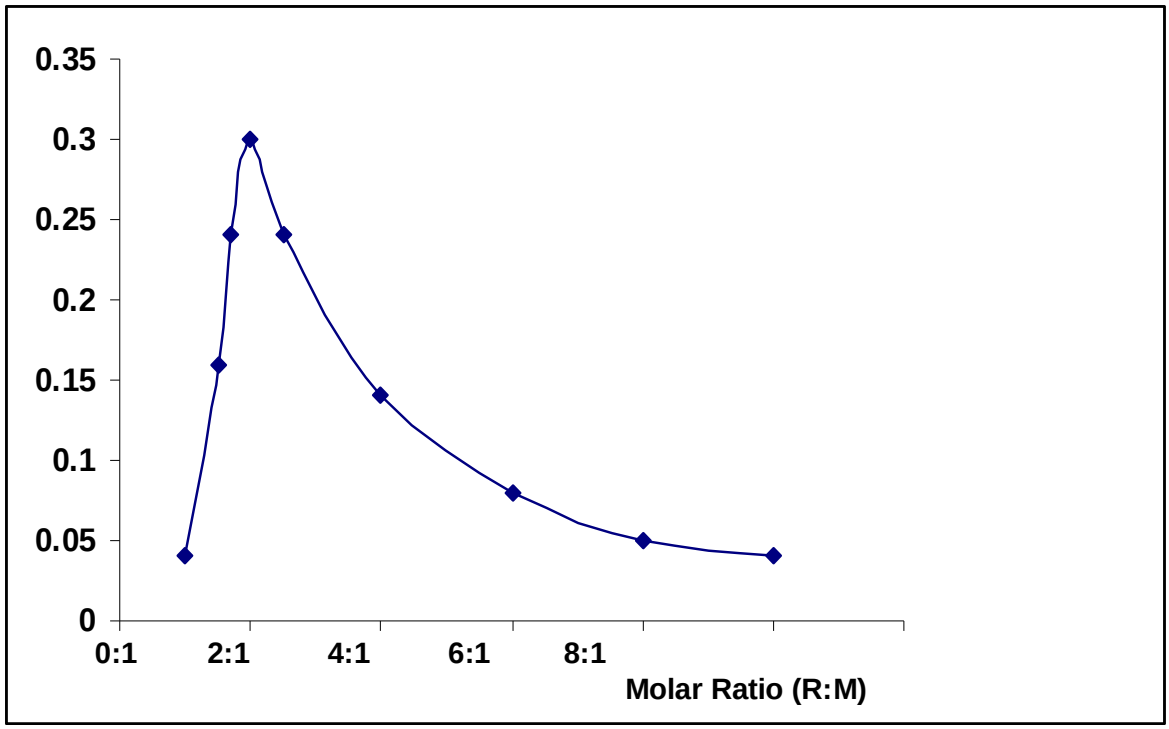


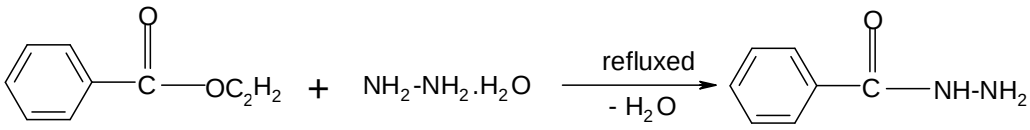
Fig. 7 .



Composition of Mo^{VI} –Bzac-BH by Molar Ratio Method

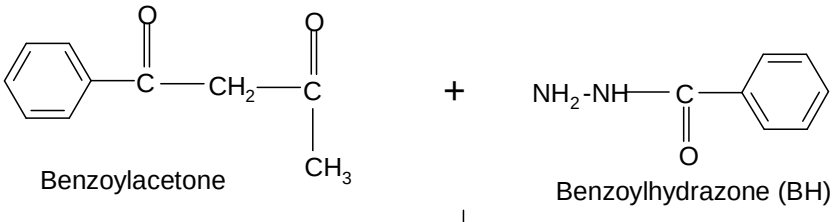
Fig. 8.

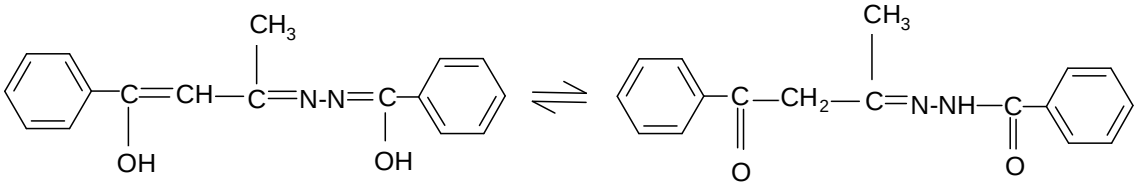
Step 1



Ethylbenzoate Hydrazinehydrate Benzoylhydrazone (BH)

Step 2





Bzac-BH$\underline{H_2}$(enol form) Bzac-BH (keto form)

Scheme 1