

Detection and Quantitative Analysis for 2-Thiobarbituric Acid Utilizing Uv-Visible Spectrophotometer

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Abstract The thiobarbiturates differ from other barbiturates in having a sulfur atom in place of the carbonyl oxygen at the C-2 position. The drug 2-thiobarbituric acid was found to be sufficiently soluble in 95% ethanol and 5% water for highly sensitive detection and assay. Analytical samples, including standards, were studied at 320 nanometer wavelength with background absorbance of the solvent correctly determined and subtracted from prepared specimens in one centimeter glass cuvettes. An ultraviolet-visible instrument Spectronic 21D was found to be suitable for this assay. The molar extinction coefficient was determined at 320nm by dissolving a known amount of 2-thiobarbituric acid into 95% ethanol and 5% water and found to be 414.9 Liter/(cm)(mole). An absorbance spectrum for 2-thiobarbituric acid is presented. The standard curve utilized a range from 0.00006267 molar to 0.004201 molar. The lowest concentration assayed was 0.01313 grams per liter (1.313E-05 grams per milliliter or 13.13 micrograms per milliliter) with the highest concentration at 0.6056 grams per liter (6.056E-04 grams per milliliter or 605.6 micrograms per milliliter). This method was found to be consistent and highly sensitive.

Keywords: 2-thiobarbituric acid, Bathyran, 2-Thio-4, 6-dioxypyrimidine, sedative, hypnotic

1. Introduction

The heterocyclic organic compound 2-thiobarbituric acid (2-thioxodihydropyrimidine-4,6(1H,5H) dione) is a sedative and hypnotic drug that is among the general depressants known as thiobarbiturates [1]. Thiobarbiturates have a sulfur atom in place of the carbonyl oxygen at the C-2 position where as oxybarbiturates retain the oxygen atom at the C-2 position. Positioning a sulfur atom to replace the carbonyl oxygen at the C-2 position incurs a higher lipid solubility than found for oxybarbiturates [1]. The substitution of a sulfur atom at the C-2 position results in higher fat solubility, short duration of action, increased hypnotic potency, accelerated metabolic degradation, and rapid onset of activity [1,2]. Thiobarbiturates have a tendency to be more toxic than oxybarbiturates along with some changes in pharmacodynamics [1]. Thiobarbiturates are utilized in medicinal applications generally as anesthetics that are administered intravenously or rectally [1,2].

Derivatives of 2-thiobarbituric acid have pharmacological and analytical applications, with other derivatives being investigated for anti-cancer and anti-viral activities [3]. Lipophilic thiobarbiturates are being studied for applications as medicaments in treatment of convulsions and anesthesiology [4]. Various derivatives of thiobarbituric acid have been shown to inhibit the enzymatic activity of the hepatitis C virus (HCV) NS5B polymerase [5]. Tyrosinase serves various roles in organisms and studies are focusing on the regulation of tyrosinase activity. Studies show that the inhibitory effect of thiobarbituric acid on tyrosinase is a reversible noncompetitive activity [6].

In addition, 2-thiobarbituric acid is utilized as a reagent for assaying malondialdehyde which is obtained from lipid peroxidation [7,8,9]. Malondialdehyde is a low molecular weight product formed from the decomposition of certain primary and secondary lipid peroxidation products [8,9]. These assays are important in handling of oxidative stress in critical care environments [9].

Therefore there is a growing need for methodologies for assaying 2-thiobarbituric acid and thiobarbiturates in general due to clinical, pharmacological, industrial, and bio-analytical use. Ultraviolet differential spectrophotometry has been utilized for measuring various thiobarbiturates previously [10]. Additionally, assay by thin layer chromatography has been successful [11]. The methodology presented in this study is an accurate and highly sensitive approach for measurement of the drug 2-thiobarbituric acid.

2. Materials and Methods

2.1 Reagents and Instrumentation

All reagents utilized as solvents were analytical grade and obtained from Sigma-Aldrich (P.O. box 14508, St. Louis MO 63178 USA). The 2-thiobarbituric acid (2-thioxodihydropyrimidine-4,6(1H,5H)-dione) utilized in this study is a stable powder, light yellow in color, and having only slight water solubility, was obtained from Eastman Organic Chemicals (P.O. Box 431, Kingsport, Tennessee, USA or AstraTech, 1 Deer Park Dr. Suite C, Monmouth Junction, New Jersey, 08852 USA).

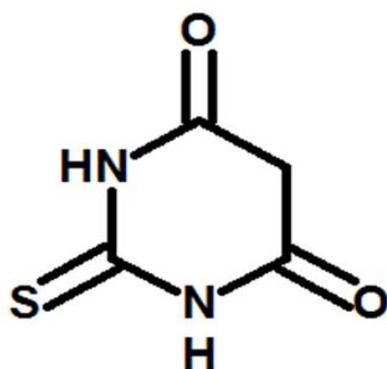
The absorbance and percent transmittance readings were collected from Milton Roy Spectronic 21D UV-Visible Spectrophotometer with stable precision 10nm

bandwidth/spectral slit-width high resolution continuous wavelength range (A.L.T., 12 Colton Road East Lyme, CT 06333). One centimeter width glass cuvettes were used throughout. The analyte 2-thiobarbituric acid dissolved readily and remained stable in 95% ethanol and 5% water. Solvent utilized throughout the project was 95% ethanol and 5% water.

2.2 Sample Analysis Parameters for Spectrophotometric Analysis

All samples of 2-thiobarbituric acid were dissolved in, and found to be highly soluble with the following solvent: 95.0% ethanol, 5.0% water. All measurements of absorbance and percent transmittance was accomplished utilizing Milton Roy Spectronic 21D UV-Vis Spectrophotometer by this solvent system. Samples of 2-thiobarbituric acid were carefully dissolved in solvent of 95 % ethanol and 5% water then examined by spectrometer in 1cm glass cuvettes. For all measurements the background absorbance of 95% ethanol and 5% water was determined first and that background absorbance subtracted from all readings involving 2-thiobarbituric acid. These proved to be efficient and permitted very highly sensitive determination of the analyte. All analytical samples and solvent mixtures were stored in glass containers and sealed air tight until use. To obtain the absorbance spectrum an amount of 0.02728 grams of 2-thiobarbituric acid was dissolved in 100mL of 95% ethanol and 5% water for scanning from 320nm to 600nm. Distilled water was utilized throughout.

The molecular properties of 2-thiobarbituric acid such as formula weight, octanol/water partition coefficient Log P, polar surface area, and Rule of 5 where stated were determined by utilizing chemical informatics system of Molinspiration (Molinspiration, Nova ulica, SK-900 26 Slovensky Grob, Slovak Republic).



2-Thiobarbituric acid

2-thioxodihydropyrimidine-4,6(1H,5H)-dione

O=C1CC(=O)NC(=S)N1

Molecular Formula = $C_4H_4N_2O_2S$

Formula Weight = 144.15

Figure 1. The molecular structure, common name, SMILES notation, and formula presented here for the sedative/hypnotic compound 2-thiobarbituric acid.

One major important facet of selecting a solvent for UV-Vis spectrometry is the requirement to dissolve the analyte, a criteria accomplished with the polar solvent of 95% ethanol and 5% water. This approach is easy to perform and convenient. In addition, the maximum absorbance peak at 320 nm is selected and falls within the efficacious ability of the Spectrometer 21D which along with the single-beam mode is the simplest optical configuration. The complete absorbance spectrum collected for 2-thiobarbituric acid in 95% ethanol and 5%

2.3 Numerical Analysis

Where indicated the numerical analysis utilizing Spearman/Kendall correlation, Kruskal-Wallis test, 95% ellipses, and Kolmogorov-Smirnov (two samples) was performed by PAST version 2.06 (copyright Hammer and Harper 1999-2011). Summary statistical analysis was also performed by Microsoft EXCEL (copyright 2010 Microsoft Corporation, Microsoft Office Professional Plus 2010). The one sample t test was performed by GraphPadInStat version 3.00 (Copyright 1992-1998 GraphPad Software Inc. (www.graphpad.com) for Windows 95, San Diego California USA).

3. Results and Discussion

The measurement of 2-thiobarbituric acid has importance due to its use in clinical environments as an anesthetic [1], bio-analytical assay of lipid peroxidation events [6,8,9], and even potential anti-viral use [3]. The methodology presented in this study which can be completed by a common simple or an advanced ultraviolet-visible (UV-Vis) spectrometer is efficacious and accurate. Some benefits of UV-Vis spectrometry analysis are: 1) low cost; 2) simple operation; and is 3) non-destructive of the analyte (e.g. the 2-thiobarbituric acid can be retrieved from solvent system). Also UV-Vis spectroscopy has been used in the clinical laboratory for many years and is almost universal in application to analytical chemistry.

The molecular structure of the sedative/hypnotic drug 2-thiobarbituric acid ($C_4H_4N_2O_2S$) is shown in Figure 1 and is distinct from the oxybarbiturates class. It has a polar surface area of 58.2 Angstroms², an octanol-water partition coefficient of Log -0.937, and zero violations of the Rule of 5 [12], which recognizes the compound as an effective oral drug.

water is shown in Figure 2 in which a strong absorbance peak at 320nm is found. This was accomplished by making a 0.001892 molar mixture of 2-thiobarbituric acid in 95% ethanol and 5% and measuring absorbance values at the indicated wavelengths settings. After complete analysis the final value for molar absorptivity (ϵ) is calculated to be 414.9 Liter/(cm)(mole).

Following identification of a strong absorbance peak within the accurate range of the single-beam Spectronic 21D a peak observed at 320nm was used for drug

determination. All sample were prepared by analytical measurement on balances capability of tenths of milligrams sensitivity. Distilled water was used with glass containers and receptacles. After measurement of solvent absorbance at 320nm, which was subtracted from

consequential measurements of test samples and standards, then various analyte preparations were examined. All test samples of the dissolved drug were maintained in air tight glass vessels prior to measurements.

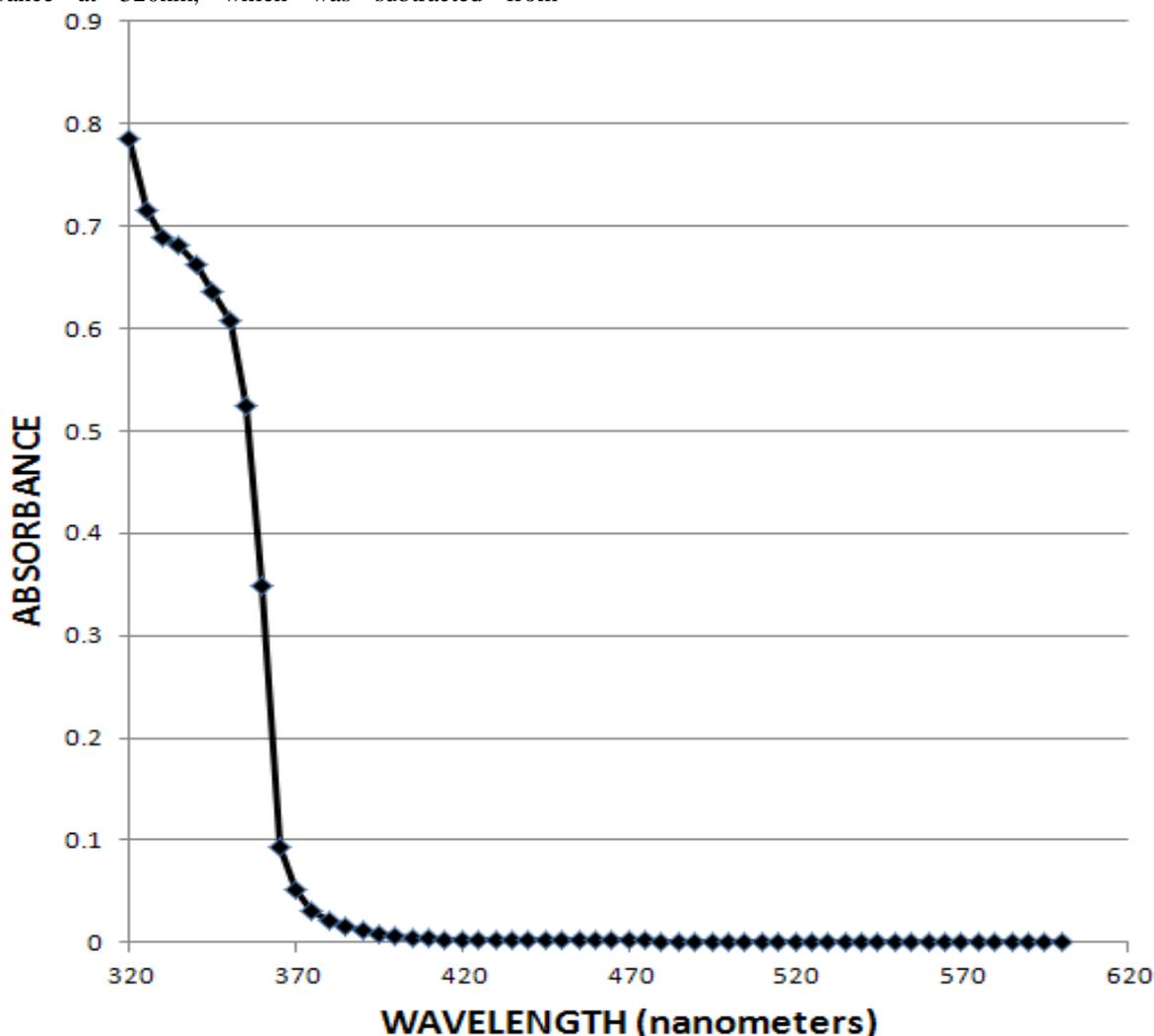


Figure 2. Absorbance spectrum of 2-thiobarbituric acid from 320 nm to 600 nm obtained in glass cuvettes and 95% ethanol with 5% water.

Use of 95% ethanol with 5% water was demonstrated to be versatile and readily dissolved all prepared test samples of the drug. Refrigeration of test samples was never necessary. In addition, this solvent system allowed facile and accurate serial dilution of any drug solution in order to bring within the absorbance scale of the single-beam instrument. Subsequent measurements of prepared mixtures for construction of a standard curve was accomplished with an outcome having a very highly linear relationship. The linear relationship was calculated to be: $y = 414.92x + 4.00E-05$. The Pearson r correlation coefficient at $r = 1.000$ and with a coefficient of determination R^2 to be 1.000, indicates that the linear model explains 100% of the variance contained in the model. The R^2 value near 1.000 indicates that a regression line fits the data extremely well.

The standard curve components were found to be extremely linear with the test samples and found on scale. Statistical analysis confirmed the excellent fit of the molarity values derived by calculation utilizing the standard curve (applying corresponding absorbance values for each test sample) with the actual molarity of the test

samples. A two-sample Kolmogorov-Smirnov (KS) test (non-parametric test having null hypothesis that both groups were sampled from populations with identical distributions). The two-sample tests for any violation of that null hypothesis to include different medians, different variances, or different distributions [13,14]. All actual molarity values of test sample data compared to the standard curve determination under two-sample KS resulted in P value of 0.9995 (so $P > 0.05$) indicating all actual test sample data when compared to the calculated values from standard curve come from populations of identical distribution. As a test for direction and strength of relationship between variables [14] the Spearman's rank correlation R_s value for standard curve members and with all test samples was determined to be $R_s = 0.9616$, indicating extremely high positive correlation. In addition the Kendall's Tau value of 0.9434 (tau a correlation coefficient similar to Spearman's correlation coefficient to show strength of association) shows that the molarity data is extremely highly ordered according to rank. The determination of 95% ellipses is a means to estimate the mean, variances, and covariances of a set of points and

calculate the two-dimensional analog of a confidence interval, referred to as confidence ellipse [15]. Here this analysis for absorbance and corresponding molarity values for both the standard curve members with all test samples

were calculated for a 95% confidence ellipses (within which 95% of the data points are expected to lie) with an outcome showing all all tests and standards numerical values included and contained within the ellipse.

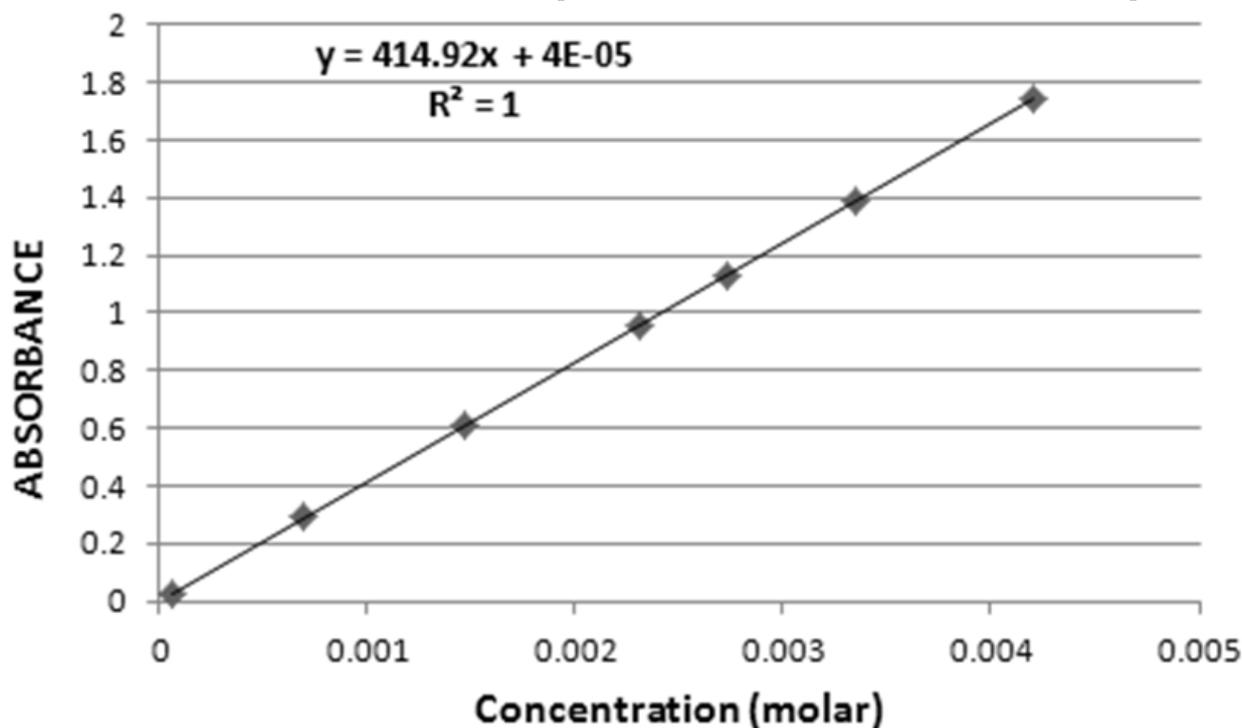


Figure 3. Standard Curve. This standard curve utilized in this study ranged from 0.00006267 molar to 0.004201 molar (a 67x wide range in molar concentration values).

Comparison of actual molarity of test samples with calculated molarity utilizing the linear standard curve was made using representative samples for determination of analyte percent recovery (see outcome presented in Table 1). Following through with statistical analysis confirms the highly consistent and accurate assay determination. Calculation of sample t test for actual and calculated molarities produces the two-tailed $P = 0.4081$ ($P > 0.05$) indicating that the means of the actual and calculated molarity populations are not different. For consideration of correlation of actual to calculated molarity the Spearman's Kendall $R_s = 0.9706$ hence indicating extremely high positive correlation. The Kendall's tau = 0.9493 showing extremely highly ordered by rank. The two sample Kolmogorov-Smirnov test is a nonparametric test that compares the cumulative distributions of two data sets [15]. Outcome for KS test provided $P = 0.9998$ (so $P > 0.05$) and indicating the calculated and actual values of molarity are from identical distributions.

The percent recovery outcome, shown in Table 1, is extremely high having a mean of 99.99%, a median at 100%, and mode at 100%. The range for percent recovery has a minimum at 99.93% and maximum at 100%. The standard deviation for percent recovery is 0.016%. The determination of 2-thiobarbituric acid is found to be consistent and with strong agreement between actual molarity determination and calculated molarity based upon linear model of standard curve constituents. The continued application of thiobarbiturates for clinical treatment of illness and application within bio-analytical assays fosters the development of additional analytical methods to monitor these compounds.

Table 1. Percent Recovery by Molarity

Calculated	Actual	Percent Recovery
0.0008411	0.0008412	99.99
0.003863	0.003864	99.97
0.000335	0.000335	100
0.0003567	0.0003567	100
0.000535	0.0005351	99.98
0.0005616	0.0005616	100
0.0005495	0.0005495	100
0.0005495	0.0005495	100
0.000576	0.000576	100
0.0005784	0.0005785	99.98
0.0005808	0.0005809	99.98
0.0005953	0.0005953	100
0.0006146	0.0006146	100
0.0006122	0.0006122	100
0.0006194	0.0006194	100
0.001663	0.001663	100
0.001767	0.001767	100
0.001974	0.001974	100
0.00207	0.00207	100
0.002179	0.002179	100
0.002335	0.002336	99.96
0.002516	0.002516	100
0.006941	0.006941	100
0.0007254	0.0007255	99.93
0.0006435	0.0006435	100
0.0006531	0.0006532	99.98
0.0006604	0.0006604	100
0.0007375	0.0007375	100
0.0006748	0.0006749	99.98
0.0006869	0.0006869	100

Conclusions

Accurate UV-Vis spectrophotometric assay is accomplished utilizing a solvent consisting of 95% ethanol with 5% water for facile solubilizing of the sedative/hypnotic 2-thiobarbituric acid. An absorbance spectrum for 2-thiobarbituric acid in the identical solvent used for assay is accomplished and shows a very strong peak at 320nm. The standard curve generated and utilized in this study ranged from 0.00006267 molar to 0.004201 molar. The lowest concentration assayed in this study was 0.01313 grams per liter (this is 1.313E-05 grams per milliliter or 13.13 micrograms per milliliter) with the highest concentration utilized at 0.6056 grams per liter (6.056E-04 grams per milliliter or 605.6 micrograms per milliliter). A standard curve described a very highly linear relationship with line calculated to be: $y = 414.92x + 4.00E-05$. The Pearson r correlation result is $r = 1.000$ and coefficient of determination to be $R^2 = 1.000$. The percent recovery was very high with minimum at 99.93% to maximum at 100% (standard deviation equal to 0.016 %). Therefore a highly accurate and facile methodology of assay for 2-thiobarbituric acid uses an alcohol-water solvent that readily dissolves this very lipophilic member of the thiobarbituric acid group of sedative/hypnotics.

Acknowledgements

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Abbreviations

UV-Vis, ultraviolet-visible; SMILES, simplified molecular-input line-entry system; KS, Kolmogorov-Smirnov test.

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