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# Optimization of the Schiff-Base Reaction of Acetylacetone with Biogenic Amines

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**ABSTRACT**

In this work, optimum conditions for the derivatization reaction of biogenic amines (histamine, tyramine, putrecine, tryptamine, phenylethylamine, cadaverine, spermidine and spermine) with acetylacetone have been determined. In this reaction, the amount of K2HPO4, reaction time, reagent amount, solvent choice and solvent amount were optimized. As a result of this study, optimum conditions were determined as amount of K2HPO4 2 g, reaction time 20 min, amount of acetylacetone 1 mL, solvent methanol and amount of solvent 10 mL.

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Biogenic amines; Acetylacetone; Derivatization; Schiff Base

## INTRODUCTION

Amines are basic nitrogenous compounds formed by substituting alkyl or aryl groups of the one, two or three hydrogen atom in ammonia. Decarboxylation of aminoacids is the most common synthesis route of foods; aromatic amines may exhibit food toxicity [1]. These amines by decarboxylation of amino acids of living organisms (bacteria) when the operating results produced are called biogenic [2]. In the presence of bacterial biogenic amine decarboxylase and suitable environmental conditions, biogenic amine formation permits bacterial growth and production of decarboxylase enzymes [3]. Biogenic amines are produced as a result of various metabolic activities of plants, animals and microorganisms. Biogenic amines containing aliphatic (putrecine, cadaverine, spermine, spermidine), aromatic (tyramine, phenylethylamine) and especially heterocyclic (histamine, tryptamine) structures are described as small molecule toxic compounds which can also be present in foods.

Biogenic amines are formed in large quantities of protein rich foods and fermented foods [4-5]. The formation of biogenic amines;

- The presence of free amino acids,
- The presence of microorganisms showing decarboxylase high enzyme activity in the medium and their number,
- Development of microorganisms
- The formation of decarboxylases depends on the presence of suitable environmental conditions such as pH and temperature [1].

It is very important to analyze and identify biogenic amines present in various foods due to their potential toxicity. Absorption intensities of the biogenic amines in the UV-Vis region are very low or not at all. Therefore, their absorption strengths need to be increased. These substances must be derivatized using an organic chelator and thus increasing the absorption intensities in the UV-Vis spectrometer becomes possible by making the resultant indirect determinations. Various derivatization reagents have been used in literature for the determination of these substances [6-22]. In this study, the use of acetylacetone reagent, previously used by Nishikawa [23] in the derivatization of primary amines, was investigated for the derivatization of

**Table 1.** Classification of biogenic amines

| | | | |
|---|---|---|---|
| *According to chemical structures* | *Aliphatic* | *Aromatic* | *Heterocyclic* |
| | *Putrecine Cadaverine Spermine Spermidine* | *Tyramine Phenylethylamine* | *Histamine Tryptamine* |
| *According to number of nitrogen* | *Monoamines* | *Diamines* | *Polyamines* |
| | *Tyramine Phenylethylamine* | *Histamine Putrecine Cadaverine* | *Spermine Spermidine* |

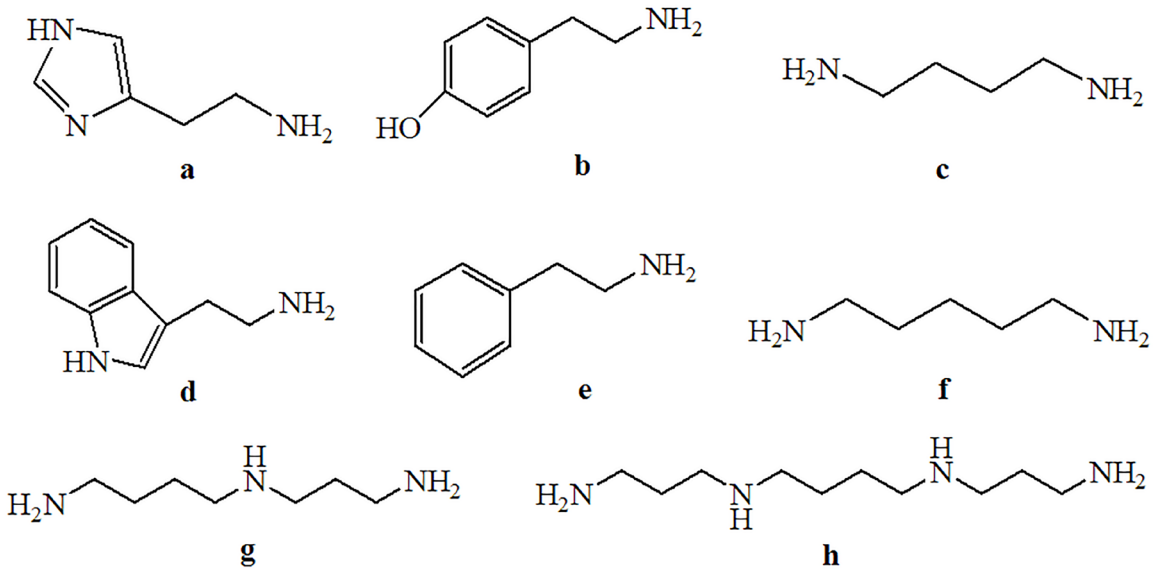


**Figure 1.** The chemical structure of biogenic amines (**a**: histamine, **b**: tyramine, **c**: putrecine, **d**: tryptamine, **e**: phenylethylamine, **f**: cadaverine, **g**: spermidine, **h**: spermine)

biogenic amines. The generic name for the derivatization reaction of biogenic amines with acetyl acetone is known as Schiff base formation reactions. In this study, optimum conditions for the derivatization reaction of the biogenic amines with acetylacetone have been determined.

The schiff bases can be represented by the general formula RCH=NR', which is obtained from the condensation of aldehydes or ketones with primary amines and also referred to as "imine" or "azomethine" compounds due to its C=N double bond as a characteristic feature in its structure [24]. R and R' is alkyl or aryl substituents. Schiff bases are also known as a good nitrogen donor ligand (>C=N-).


**Figure 2.** The formation reaction of Schiff base (R-R'-Alkyl or Aryl)

## MATERIALS AND METHODS

### Chemical and Reagents

Biogenic amine standards (histamine, tyramine, putrecine, tryptamine, phenylethylamine, cadaverine, spermidine, spermine), were obtained from Sigma–Aldrich. Acetylacetone was supplied by Merck. Dipotassium hydrogen phosphate ($K_2HPO_4$) was purchased from Merck. Acetone, acetonitrile, ethanol, methanol and tetrahydrofuran were obtained from Sigma-Aldrich (HPLC-grade).

### Apparatus

The UV–Vis absorbance spectra were recorded at using a GENESYS™ 10S Thermo Scientific Perkin Elmer spectrophotometer, equipped with a 1 cm path length cell, controlled by a personal computer. This equipment has a degasser system (WiseClean). Mettler Toledo MA 235 pH/ion analyzer with Hanna HI 1332 Ag/AgCl combined glass electrode was used for pH measurements.

### Preparation of standard solutions of biogenic amines

Stock solutions of histamine, tyramine, putrecine, tryptamine, phenylethylamine, cadaverine, spermidine, spermine were prepared by dissolving each biogenic amine in 10 % (v/v) methanol/water. The diluted solutions were taken from this stock solution and diluted to the desired concentration in the same mixture. Stock solutions were kept +4 $^0$C and stored in the dark. Water-methanol mixture was used for the further dilutions of the solutions of biogenic amines.

### Derivatization Process

The final concentration of each biogenic amine solution was taken from stock solution as 1x $10^{-4}$ M. To this, methanol, $K_2HPO_4$ and derivatization reagent acetylacetone were added in a certain amount and volume 100 mL completed. The mixture was allowed to stand in the dark and then their UV-Vis spectra were taken against the blank solution. Biogenic amines, the effect of all parameters of the reaction to optimize the derivatization reaction which is converted into schiff base using acetylacetone reagent were examined individually. For this purpose, parameters such as $K_2HPO_4$ amount, reaction time, acetylacetone amount, solvent effect and solvent amounts were investigated and optimum conditions were obtained for each.

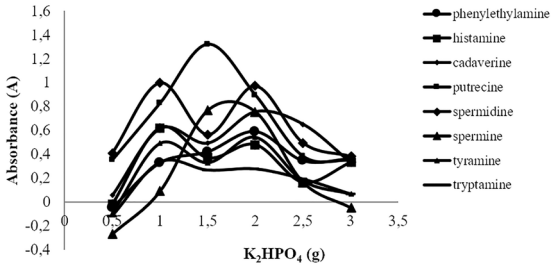


**Figure 3.** The effect of the amount of $K_2HPO_4$ on the derivatization reaction of biogenic amines with acetylacetone

## RESULTS AND DISCUSSION

### Effect of the Amount $K_2HPO_4$

The reaction of biogenic amines with acetylacetone takes place in mildly basic medium. For this purpose, $K_2HPO_4$ was added in different amounts and the changes in absorption were evaluated according to spectrophotometric measurement results. For this, an appropriate amount of each biogenic amine was added to a solution containing 10 mL methanol, different amounts of $K_2HPO_4$ (0.5; 1.0; 1.5; 2.0; 2.5 and 3.0 g) and 1 mL of acetylacetone. The mixture was then diluted to 100 mL with ultrapure water. The mixture was kept in dark for 40 min to complete the reaction. The absorption changes of different amounts of $K_2HPO_4$ obtained on the spectra are as shown in Figure3.

The amount of $K_2HPO_4$ from which the highest absorptions were obtained was determined to be 1 g and 1 g $K_2HPO_4$ was used for all derivatization reaction with acetylacetone of biogenic amines throughout the study.

### Effect of Time

To determine the extent to which the reaction of the derivatives of the biogenic amines with acetylacetone reactives was carried out, the solutions in which the derivatives were obtained at certain time intervals were left in the dark and the UV-Vis spectra were taken. Reaction time was optimized taking into account the absorption intensities in the spectra. An appropriate amount of each biogenic amine was added to a solution containing 10 mL methanol, 1 g $K_2HPO_4$ and 1 mL of acetylacetone. The mixture was then diluted to 100 mL with ultrapure water. Each solution was kept in the dark at 10, 20, 30, 40, 50 and 60 min intervals and UV-Vis spectrum were taken. The absorption changes obtained at different times in the spectrum are the same as in Figure 4.

The reaction time at which the highest absorptions were obtained was determined as 20 min. During the study, the solutions for the derivatization reaction of biogenic amines with acetylacetone were left in the dark for 20 min.

### Effect of the Amount Acetylacetone

To optimize the amount of acetylacetonate used as the reagent for the derivatization of biogenic amines, the optimum amount was determined by adding acetylacetone medium at various ratios. An appropriate amount of each biogenic amine was added to a solution containing 10 mL methanol, 1 g $K_2HPO_4$ and different amounts of acetylacetone (0.6; 0.8; 1.0; 1.2 and 1.4 mL). The mixture was then diluted to 100 mL with ultrapure water. The mixture was kept in dark for 20 min to complete the reaction. The absorption changes of different amounts of acetylacetone obtained on the spectra are as shown in Figure 5.

The amount of acetylacetone in which the highest absorption intensity was obtained was determined to be 1 mL and this value was used for the derivatization reaction throughout the study.

### Effect of Different Solvents

The optimal solvent was determined using various solvents to reveal the solvent to be used in the derivatization reaction of the biogenic amines and the effect on the reaction. For this purpose, different solvent containing derivatization reactions were performed for each compound. An appropriate amount of each biogenic amine was added to a solution containing 10 mL solvent (methanol, ethanol, acetone, acetonitrile and tetrahydrofuran) 1 g $K_2HPO_4$ and 1 mL of acetylacetone. The mixture was then diluted to 100 mL with ultrapure water. The mixture was kept in dark for 20 min to complete the reaction. The absorption changes of

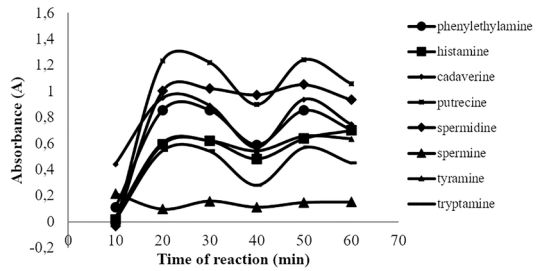


**Figure 4.** The effect of time on the derivatization reaction of biogenic amines with acetylacetone

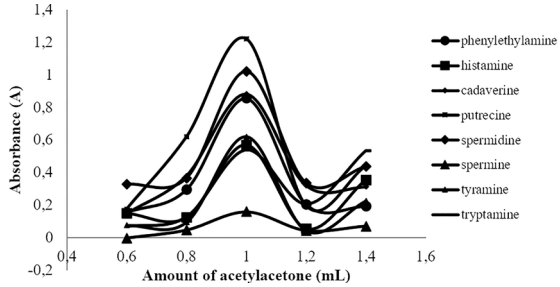


**Figure 5.** The effect of the amount of acetylacetone on the derivatization reaction of biogenic amines with acetylacetone

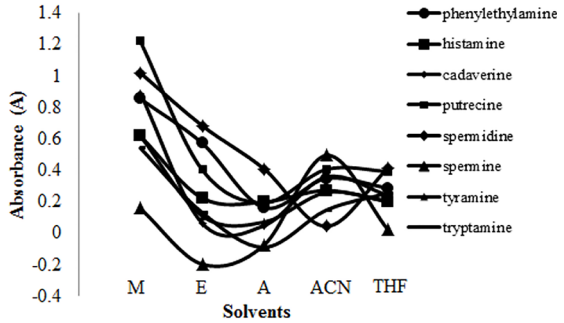


**Figure 6.** The effect of solvent on the derivatization reaction of biogenic amines with acetylacetone (M: methanol, E: ethanol, A: acetone, ACN: acetonitrile, THF: tetrahydrofuran)

different solvents obtained on the spectra are as shown in Figure 6.

It is seen that the solvent in which the highest absorptions are obtained is methanol. Therefore, the methanol-water mixture was used as this solvent during the derivatization of the biogenic amines with acetylacetone.

### Effect of Solvent Volume

After determining that the most suitable solvent for the derivatization reaction of the biogenic amines with acetylacetone was methanol, the effect of this solvent volume on the absorption intensity was investigated. An appropriate amount of each biogenic amine was added to a solution containing different volumes of methanol (6, 8, 10, 12 and 14 mL), 1 g $K_2HPO_4$ and 1 mL of acetylacetone. The mixture was then diluted to 100 mL with ultrapure water. The mixture was kept in dark for 20 min to complete the reaction. The absorption changes of different volumes of methanol obtained on the spectra are as shown in Figure 7.

It is seen that the solvent in which the highest absorptions are obtained is 10 mL of methanol. For this reason, 10 mL of methanol was used during the derivatization of biogenic amines.

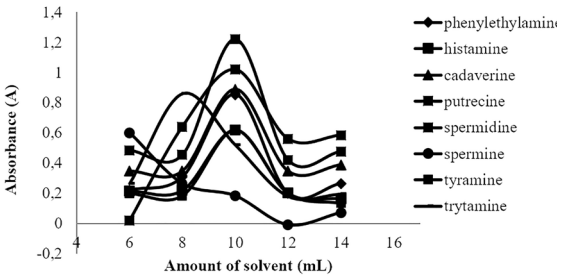


**Figure 7.** The effect of the volume of methanol on the derivatization reaction of biogenic amines with acetylacetone

## CONCLUSION

In this study, the derivatization reaction of biogenic amines (phenylethylamine, histamine, cadaverine, putrecin, spermidine, spermine, tyramine and tryptamine) with acetylacetone reagent was optimized. The amount of $K_2HPO_4$, the reaction time, the amount of acetylacetone, the solvent and the solvent amount were determined in the optimization studies. This reaction was carried out by adding 1 g of $K_2HPO_4$, 10 mL of methanol, 1 mL of acetylacetone on the biogenic amine solution taken at a given concentration and diluting to 100 mL with a pure and standing for 20 minutes in the dark to complete the reaction. The end result is that the biogenic amines are converted to schiff bases.

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