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The Reliability of Jung-Biggs-Moorhead Method for Cholesterol Determination in the Presence of Ergosterol

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In the present study, the reliability of Jung-Biggs-Moorhead method for cholesterol determination has been tested in the presence of another steroid, ergosterol. It was observed that presence of ergosterol interferes with the absorbance measurement of cholesterol. When both steroids are present in the sample maximum absorption wavelength are not shifted by the mutual interaction but absorbance values are changed considerably. Therefore this method may be used only for qualitative bu not quantitative analysis for cholesterol in the presence of ergosterol.

Introduction

Steroids are important biological regulators, such as male and female sex hormones, D vitamins etc.[1–3]. The two common steroids that can be found in foodstuffs are cholesterol and ergosterol. Note that although chloresterol is widely distributed in the animal kingdom, ergosterol plays a simlar role in the plant kingdom[1–3]. The two common steroids that can be found in foodstuffs are cholesterol and ergosterol. Note that although chloresterol is widely distributed in the animal kingdom, ergosterol plays a similar role in the plant kingdom[1–3]. These compounds can be extracted from animal and plant tissues by using an organic solvent [4–5] (e.g. ether, methanol, etc.). There are several methods for determination of cholesterol, ergosterol and other sterols[6–9], such as GC/MS, HPLC, and Jung-Biggs-Moorhead[10]. In the UV-region, the maximum absorbances of cholesterol and ergosterol occur at 202 nm and 272-282 nm, respectively.

The Jung-Biggs-Moorhead method has been used for routine analysis, especially for the quantitative determination of serum cholesterol level[10]. Since one can apply this method by using a simple colorimeter, it is a very practical method. Therefore we have intended to study the validity of this method in the presence of ergosterol.

Materials and Methods

The chemicals used as standards in this study are ergosterol and cholesterol. Both were purchased from Sigma Chemical Co. The method, first proposed by D. H. Jung, H. G. Biggs, and W. R. Moorhead [10] in 1975 has been used with some modifications. In the present study three tubes were used, one for cholosterol, one one for ergosterol and the third for blank, instead of four tubes suggested in the Jung-Biggs-Moorhead method labeled unknown, standard, control and blank. Procedure is as follows;

1- Tubes were labeled "cholesterol" and "ergesterol".

2- 3.5 ml of ferric acetate/uranyl acetate reagent was added to each tube.

3- With a capillary pipette 0.5 ml of the appropriate sample (ca.10^{-5} M) was added to the tubes, mixed and allowed to stand for 10 min.

4- To additional tubes, labeled "cholesterol", "ergesterol", and "blank", 2.0 ml of the sulfuric acid reagent was added.

5- To these tubes, except "blank" tube, 3 ml of each corresponding solutions prepared in step 3 was transferred and layered above the sulfuric acid reagent by allowing them to flow down the sides of the tubes. This step was done carefully to prevent mixing.

6- To the tube marked blank", 3.0 ml of the ferric acetate/uranyl acetate solution was transferred and layered.

7- As quickly as possible, the contents of each tube were mixed by continuous shaking until the reaction was completed. Then allowed to cool to room temperature.

8- The absorbance of each solution was measured at 430, 490, 520, 540 and 580 nm after adjusting the colorimeter to zero absorbance with the blank solution in the light-path. Also, the whole spectrum was obtained in the visible region by using a UV-VIS spectrophotometer.

Cholesterol and ergosterol, individiually and mutually, in various concentrations, were used to determine he reliability of the Jung-Biggs-Moorhead method. Triplicate runs were performed for each concentration.

Reagents

1- Ferric acetate/Uranyl acetate Reagent:

Five hundred mg of ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) were dissolved throughly in the mixture of 10 ml distilled water and 3 ml of 28% ammonia solution and centrifuged. The supernatant solution was decanted and the precipitate was washed several times with distilled water until the supernatant gives a negative test for chloride. After the last centrifugation the precipitate was dissolved in acetic acid and transferred into a 50 ml volumetric flask. 0.1 gram of uranyl acetate ($UO_2(CH_3COO)_2 \cdot 2H_2O$) was dissolved and completed to 50 ml with glacial acetic acid.

2- Sulfuric acid / Ferrous sulfate Reagent :

1.0 gram of ferrous sulfate was dissolved in 2.5 ml of distilled water, while stirring 10 ml of glacial acetic acid and 10 ml of concentrated sulfuric acid were added and transferred into a 100 ml volumetric flask, cooled to room temperature and completed to 100 ml by adding concentrated sulfuric acid.

Both reagents were bottled in dark brown bottles and left for a day before using.

Results and Discussion

Since our purpose initially was to apply the Jung-Biggs-Moorhead method in the determination of cholesterol and ergosterol contents of archaeological samples, firstly the method had to be studied to see whether it was suitable for this purpose or not. Therefore, in the present study the standard solutions with known concentrations were prepared then wavelength of absorptions of pure cholesterol, pure ergosterol, and the mixed solutions of these two compounds were measured by applying Jung-Biggs-Moorhead method. The Vis-spectra of cholesterol and ergosterol were obtained by a UV-VIS spectrophotometer (Shimatzu UV 2100 UV-VIS) using Jung-Biggs-Moorhead method. The results revealed that the maximum absorbance for cholesterol was at 560 nm, whereas ergosterol exhibited a broad peak in the region of 443-450 nm (Figures 1 and 2).

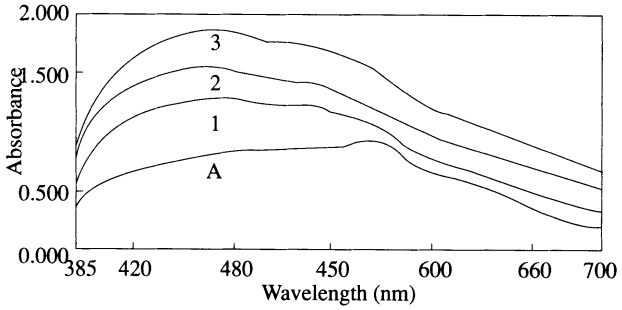

Figure 1. Wavelength spectra of mixed solutions of ergosterol and cholesterol in Jung-Biggs-Moorhead medium. Molarity of cholesterol is kept constant at 20 $\times 10^{-5}$ M. The molarity of ergosterol is: 4×10^{-5} M (pattern 1); 9×10^{-5} M (pattern 2); 14×10^{-5} M (pattern 3). Pattern A is the spectrum of cholesterol only (20×10^{-5} M).

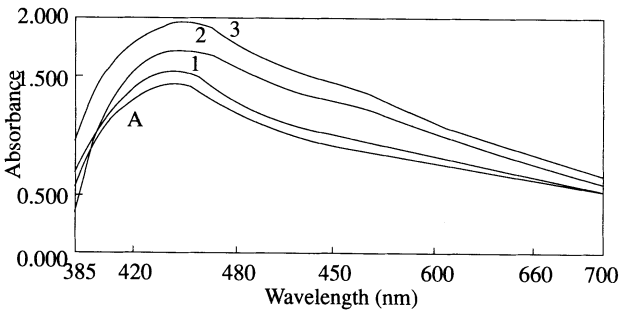

Figure 2. Wavelength spectra of mixed solutions of ergosterol and cholesterol in Jung-Biggs-Moorhead medium. Molarity of ergosterol is kept constant at 20×10^{-5} M. The molarity of cholesterol is; 4×10^{-5} M pattern 1); 9×10^{-5} M (pattern 2); 14×10^{-5} M (pattern 3). Pattern A is the spectrum of ergosterol only (20×10^{-5} M).

The absorbance values of the pure and mixed solutions of these two compounds were obtained by the method of Jung-Biggs-Moorhead. The measured absorbance values of the mixtures were found to be higher than the absorbance values of the pure solutions. The maximum wavelengths of absorbance for the mixtures are in 540 nm and 490 nm. According to these results, in the mixed solutions the absorbance values change but the values of absorption wavelengths practically do not change compared to the pure solutions of cholesterol and ergosterol.

However, the interference of the compounds was measured by keeping the concentration of cholesterol constant in the mixed solution, and changing the concentration of ergosterol. It was found that the interference was less when the concentration of ergosterol was low and practically there was no interference when 1×10^{-5} M ergosterol was present in the mixed solution. Therefore, 1×10^{-5} molarity can be accepted as the limit value for interference of ergosterol. Below this limit there is no interference, in the

Jung-Biggs-Moorhead method.

Although the study of interference was carried out by measuring the wide wavelength spectrum in the visible region using a UV-VIS spectrophotometer, firstly the wavelength spectrum of 20×10^{-5} M cholesterol, 20×10^{-5} M, ergosterol, and the mixed solution of cholesterol and ergosterol having 10×10^{-5} M concentration were obtained. The mixture showed the maximum absorbances at the similar wavelengths but with higher values. It was found that, although the molarities were decreased by half in the mixture but the absorbance values were found to be higher in comparison with absorbance values of separate solutions of ergosterol and cholesterol. The increase in the absorbance value for ergosterol (0.84) was twice that of the increase in the absorbance value for cholesterol (0.41). For this reason the ergosterol peak was recognized easier than the cholesterol peak in the mixture solutions having different concentrations of components.

Figures 1 and 2 show the wavelength spectra of the mixed solutions in which the concentration of one of the components was kept constant and the concentration of the other was varied. As shown in Figure 1, pattern A is for pure cholesterol solution (20×10^{-5} M) and in all three mixtures the molarities of cholesterol were 20×10^{-5} M. The ergosterol molarities were changed from 4×10^{-5} M to 9×10^{-5} M and to 14×10^{-5} M. In the pattern shown in Figure 2, just the reverse was done, where the molarity of ergosterol was kept constant at 20×10^{-5} M and the molarity of cholesterol was varied from 4×10^{-5} M to 9×10^{-5} M and to 14×10^{-5} M. As it can be seen in the pattern of the solution (4×10^{-5} M cholesterol + 20×10^{-5} M ergosterol) in Figure 2, the cholesterol peak is not distinguishable by the method of Jung-Biggs-Moorhead.

Conclusion

The present study reveals that the interference of cholesterol and ergosterol in the method of Jung-Biggs-Moorhead depends upon the ratio of these two compounds in the mixture.

Therefore, the presence of ergosterol causes an erroneous determination of the amount of cholesterol, so that the observed value tends to be more than the real one. Also, ergosterol prevents the recognition of the cholesterol peak if the ratio of ergosterol to cholesterol is 5 :1 or more. On the other hand, ergosterol can be qualitatively determined in the samples even in the presence of cholesterol, if the former sterol has a concentration higher than 1.10^{-5} M.

References

1. A. White, P. Handler, E. L. Smith, **Principles of Biochemistry,** McGraw-Hill, New York, 1968.
2. A. C. Guyton, **Text Book of Medicinal Physiology,** W. B. Sounders Co., Philadelphia, 1981.
3. F. S. Greenspan, P. H. Forsham, **Basic and Clinical Endocrinology,** Lange, Los Altos, California, 1983.
4. M. W. Dawis and R. T. Lamar, **Soil Biol. Biochem, 3,** 189-198 (1992).
5. M. O. Gessner, M. A. Bauchrawitz and M. Escautier, **Microb. Ecol. 22,** 285-291 (1991).
6. C. Arnezerder, W. Koliander and W. A. Hampel, **Analytica Chemica Acta, 225,** 129-136 (1989).
7. R. J. Rodriguez and L. W. Parks, **Analytical Biochemistry 19,** 200-204 (1982).
8. I. R. Hunter, K. M. Walden, E. Heftmann, **J. of Chromatogrophy 153,** 57-61 (1978).
9. G. W. Patterson, **Analytical Chemistry, 43**, 10, (1971).
10. D. H. Jung, H. G. Biggs and W. R. Moorhead, **Clinical Chemistr, 10,** 1526-1530, (1975)