First Derivative Spectrophotometric Determination of Cholesterol and Triglyceride in Human Serum

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Abstract: Cholesterol is present in tissues and plasma lipoproteins either as free cholesterol or combined with a long chain fatty acid as cholesterol ester. Triglycerides are the simplest lipids constructed from fatty acids and glycerol. Derivative spectrophotometric techniques were used for determination of individual and simultaneous determination of cholesterol and triglyceride. First derivative spectrophotometric technique was used for determination of cholesterol and triglyceride in serum samples in the concentration ranges (10 – 100 µg/ml) for cholesterol with correlation coefficient 0.9983 and detection limit 2.9 µg/ml with good precision and accuracy basing upon measuring height at 218 nm depending upon the values of the relative standard deviation percentage (RSD %) and the relative error percentage (Error %) for ten replicate measurements of three different concentration and (15 – 150 µg/ml) for triglyceride with correlation coefficient 0.9972 and detection limit 5.5 µg/ml with good precision and accuracy basing upon measuring peak - to - baseline at 242 nm depending upon the values of the relative standard deviation percentage (RSD %) and the relative error percentage (Error %) for ten replicate measurements of three different concentration.

Keywords: Derivative, Determination, Cholesterol and Triglyceride

1. Introduction

Cholesterol is a structure containing 27 carbons, commonly found as the component in cell membrane. Cholesterol is present in tissues and plasma lipoproteins either as free cholesterol or combined with a long chain fatty acid as cholesterol ester. In humans, cholesterol is obtained directly from diet and also biosynthesized from acetate via squalene in the liver. Cholesterol is only found in animal products, the main sources of dietary cholesterol are meat, poultry, fish and dairy products. Organ meats such as liver are especially high in cholesterol content, while foods of plant origin contain no cholesterol. A proximately half the cholesterol of the body arises by synthesis about (500 mg /dl) and the remainder is provided by the average diet. Animal fats and vegetable oils are triglycerides. Triglycerides are the simplest lipids constructed from fatty acids and glycerol Triglycerides make up the bulk of ingested lipid and they are hydrolyzed to fatty acid and glycerol by lipases in the gut. Re-esterification then takes place in the gut mucosa with the formation of triglycerides once more. The fat is then transported to the blood via the lymphatic system in the form of chylomicrons. The composition of human diet plays an important role in the management of lipid and lipoprotein concentration in the blood. Triglyceride level in human less than (150 mg/dl), (150 – 199 mg/ dl) borderline, (200 – 499 mg/dl) high and (500 mg /dl very high). Triglycerides along with cholesterol are the plasma lipid of the most interest in the diagnosis and management of lipoprotein disorder.

This paper describes 1st derivative spectrophotometric method for determination of cholesterol and triglyceride in human serum and compare with Biolabo kit and biolis automated instrument.

2. Materials and Methods

Apparatus

Spectral measurements were carried out on a BIO-TIK UV/Visible spectrophotometer model: J643002, sn 1025, by using 1-cm quartz cells Centrifuge. SHIMADZU UVProbe data system program (Version 2.43) equipped with dual core processor laptop having 3.0 RAM (Windows 7 operating system) was used for recording the1st derivative spectra for each one cholesterol and triglyceride solutions in the derivative spectrophotometric determination of binary systems.

Reagents

Chemicals were used are of the highest purity available.

3. Results and Discussion

Fig. 1 revealed that 1st derivative spectrum of cholesterol solution is simple and gave best results of highest accuracy and detection limit. Graphically basing upon the height of 1st derivative spectra relation between the concentrations of cholesterol and some signals or features of the 1st derivative spectra of cholesterol has been obtained.

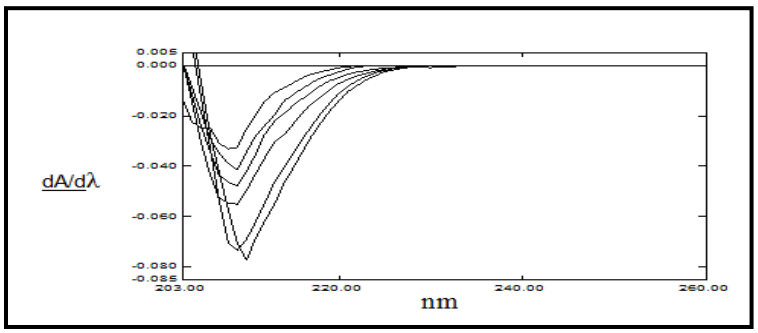

Fig. 1: 1st derivative spectra of cholesterol containing (10 –100) µg/ml

Fig. 2 1st order derivative spectrum of triglyceride solution is simple and gave best results of highest accuracy and detection limit.

Graphically basing upon techniques (peak – to – baseline) of 1st derivative spectra relation between the concentrations of triglyceride and some signals or features of the 1st derivative spectra of triglyceride have been obtained.

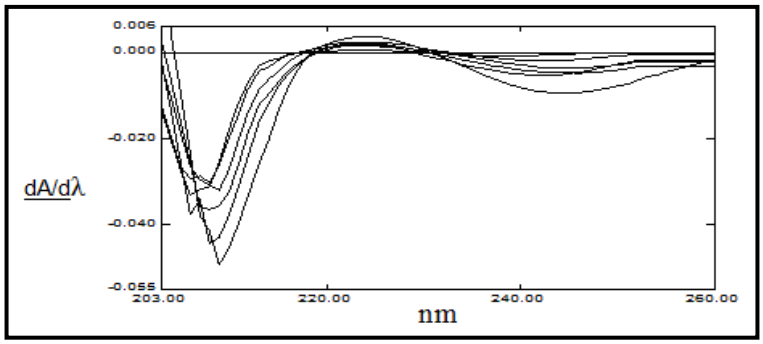

Fig. 2: 1st derivative spectra of triglyceride solution containing (50 -150µg/ml)

Simultaneous first derivative spectrophotometric determination of cholesterol and triglyceride using zero – cross technique

Fig. 3 shows absorption spectrum of (50 µg/ml) of cholesterol solution curve (a), absorption spectrum of (50 µg/ml) of triglyceride solution curve (b), absorption spectrum of the mixture of (50 µg/ml) of each one of cholesterol and triglyceride curve (c). It is obvious that there is a strong overlap of the spectrum of cholesterol and triglyceride therefore the determination basing upon zero order absorption spectra measurements, when present in the same solution is very difficult due to strong over lopping and interfering between their spectra.

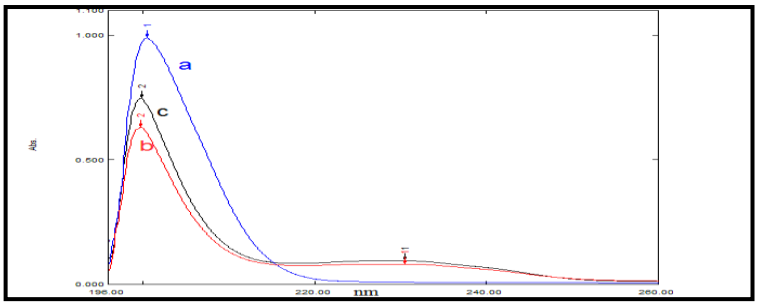

Fig. 3: Normal absorption spectrum of cholesterol (a), triglyceride (b) and their mixture (c)

As shown in Fig. 1 and 2, 1st derivative spectrum of triglyceride solution undergoes zero absorption at wavelength 218 nm, while cholesterol solution has absorption at this point. From this point of view zero cross technique was used for determination of cholesterol at the presence of triglyceride in the solution. Also at 242 nm, triglyceride solution has negative valleys, while cholesterol solution has no absorption, therefore basing upon amplitude measurements of these negative peak to baselines, triglyceride solution was determined at the presence of cholesterol in the solution. Thus, simultaneous determination of triglyceride and cholesterol was performed with 1st derivative spectrophotometric method.

Simultaneous First derivative spectrophotometric determination of cholesterol and triglyceride

Fig. 4 different mixture solution of triglyceride and cholesterol were prepared in a way that the concentration of triglyceride kept constant (30 µg/ml) with varying concentration of cholesterol for determination of cholesterol in the presence of triglyceride and the same condition for recording of the 1st derivative spectra of triglyceride and cholesterol were applied. The results indicated that when concentration of triglyceride kept constant, the concentration of cholesterol is directly proportional

with the height at 218 nm (zero crossing point of triglyceride) over the concentration range of (10 – 100 µg/ml), with 2.2 µg/ml of detection limit and r = 0.9971.

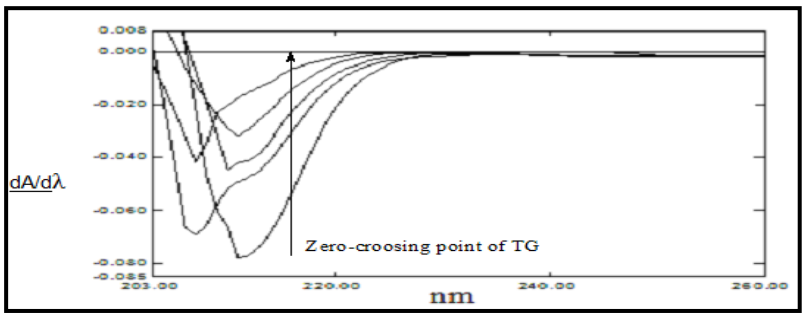

Fig. 4: 1st derivative spectra of mixtures containing (10 – 100 µg/ml) cholesterol and 30 µg/ml triglyceride

Simultaneous First derivative spectrophotometric determination of triglyceride and cholesterol

Fig. 5 different mixture solution of triglyceride and cholesterol were prepared in a way that the concentration of cholesterol kept constant (30 µg/ml) with different concentration of triglyceride, and the same conditions for recording the 1st derivative spectra of triglyceride and cholesterol were applied, in order to determine triglyceride in the presence of cholesterol in the same solution. The results showed that when the concentration of cholesterol kept constant the concentration of triglyceride in linear proportion with peak – to – baseline amplitude at 242 nm (negative peaks) over the concentration range of (15 –150 µg/ml), with 6.6 µg/ml of detection limit, and r =0.997.

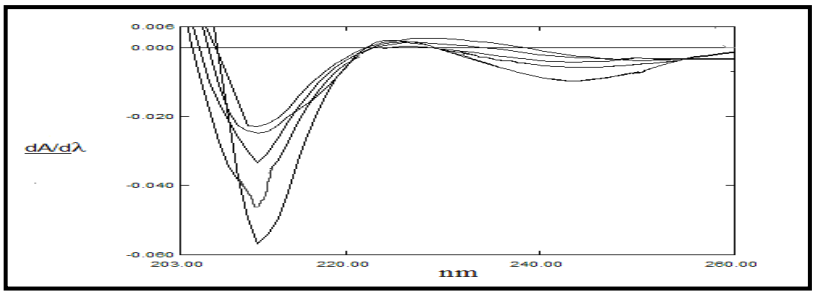

Fig. 5: 1st derivative spectra of mixtures containing (15 – 150 µg/ml) triglyceride and 30 µg/ml of cholesterol

4. Conclusion

The proposed derivative spectrophotometric method for individual and simultaneous determination of binary mixture (cholesterol and TG) are relatively easy (just recording the normal absorption spectrum of the compounds under study and using of SHIMADZU UVProbe software to obtain different orders of the absorption spectra). Simultaneous determination of cholesterol and triglyceride done with 1st derivative spectra basing upon zero-crossing technique without any separation presses. The results obtained by proposed methods are in good agreement when compared with the results obtained by bilabo kit and biolis automate instrument.

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