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Graduation project

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5th stage students

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Analysis of Omega-3 fatty acids supplement

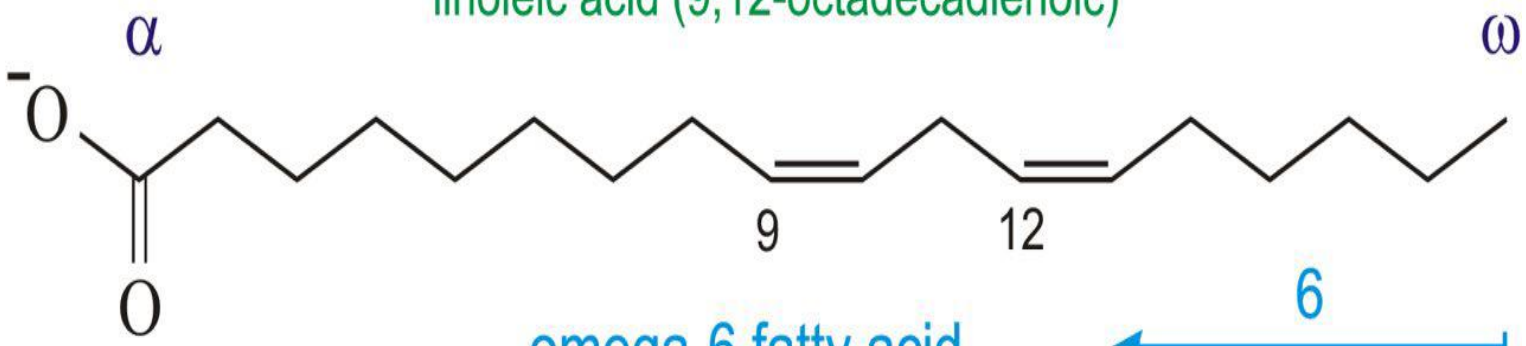
Introduction :

- Omega-3 fatty acids are considered essential fatty acids ،They are necessary for human health, but the body can't make them. You have to get them through food.
- Omega-3 fatty acids are found in fish, such as salmon, tuna, and halibut, other sea foods including algae and krill, some plants, and nut oils. Also known as polyunsaturated fatty acids (PUFAs).
- omega-3 fatty acids play a crucial role in brain function, as well as normal growth and development. They have also become popular because they may reduce the risk of heart disease.
- and behavioral function.

- Research shows that omega-3 fatty acids reduce inflammation and may help lower risk of chronic diseases such as heart disease, cancer, and arthritis. Omega-3 fatty acids are highly concentrated in the brain and appear to be important for cognitive (brain memory and performance)

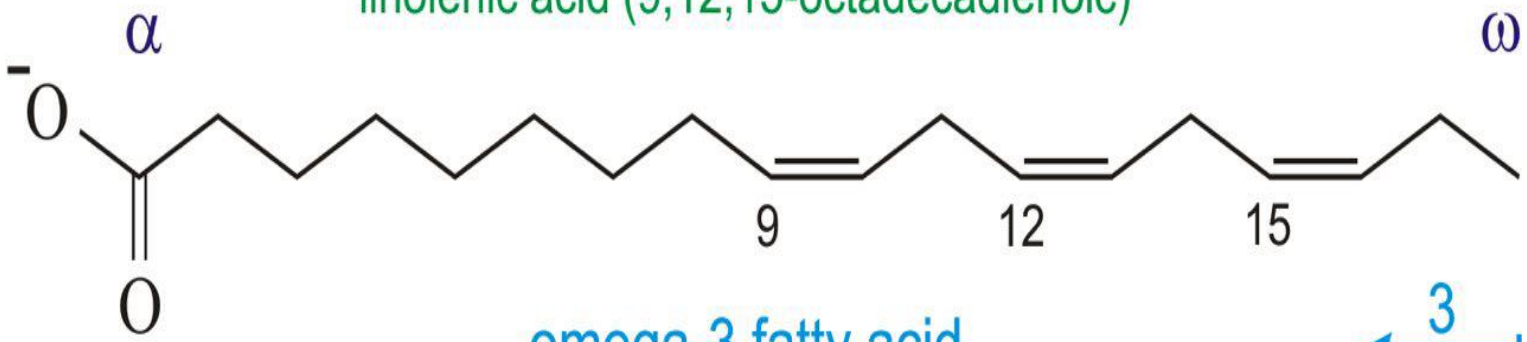
- Symptoms of omega-3 fatty acid deficiency include fatigue, poor memory, dry skin, heart problems, mood swings or depression, and poor circulation. It is important to have the proper ratio of omega-3 and omega-6 (another essential fatty acid) in the diet.
- In fact , studies suggest that higher dietary omega-6 to omega-3 ratios appear to be associated with worsening inflammation over time and a higher risk of death among hemodialysis patients.
- The Mediterranean diet, on the other hand, has a healthier balance between omega-3 and omega-6 fatty acids ,Many studies have shown that people who follow this diet are less likely to develop heart disease.
- The Mediterranean diet emphasizes foods that are rich in omega-3 fatty acids, including whole grains, fresh fruits and vegetables, fish, olive oil, garlic,

linoleic acid (9,12-octadecadienoic)



omega-6 fatty acid

linolenic acid (9,12,15-octadecadienoic)



omega-3 fatty acid

Dietary Sources :

- Fish, plants, and nut oils are the primary dietary sources of omega-3 fatty.

Available Forms :

- Omega 3 can be taken in the form of soft gel capsules either alone or in combination with other oils and vitamins.

How to take it :

- **Children (18 years and younger):**

There is no established dose for children.

- **Adults :**

DO NOT take more than 3 grams daily of omega-3 fatty acids from capsules.

Benefits of using Pharmaceutical Grade Omega-3 Fish Oil

- Reduces migraine
- Relief from joint pains and menstrual pains
- Controls blood pressure
- Reduces weight
- Improves memory function
- Improves eye sight

Omega-3 Fish Oil Side Effects Are There Any?

- Most Omega-3 fish oils are safe
- Some experience Omega-3 fish oil side effects but this is almost always due to the quality of the omega-3 supplement being used
- Poorly processed Omega-3 fish oils also contain mercury and other contaminants that can cause the side effects

Precautions :

- Because of the potential for side effects and interactions with medications, you should only take dietary supplements only under the supervision of a knowledgeable health care provider.
- Omega-3 fatty acids should be used cautiously by people who bruise easily, have a bleeding disorder, or take blood-thinning medications, including warfarin (Coumadin), clopidogrel (Plavix), or aspirin.
- High doses of omega-3 fatty acids may increase the risk of bleeding, even in people without a history of bleeding disorders, and even in those who are not taking other medications.
- Fish oil can cause gas, bloating, belching, and diarrhea.

There are several methods of Omega-3 analysis :

- **Such as :** The content of omega-3 in the fish oil capsules have been determined by alkalimetrie titration method. This method has been done in two ways.
  - Firstly, fatty acids were oxidized by KMnO4 using H2SO4 as catalyst.
  - Secondly, propionic acid as the result of oxidation was separated by distillation. Furthermore, distillated propionic acid was titrated.
- Precision, accuracy, repeatability, and reproducibility of the research were good enough for further development as a routine method for determination of omega-3 content.

IR(infrared) spectrophotometry :

- is a preferred and reliable method of analysis for the determination of fatty acids in both animal fats and partially hydrogenated vegetable oils.
- Fatty acid composition can be measured through infrared light absorption values and calculated using a comparison method in relation to the trans content levels.
- These measurements can be obtained through colorimetric differentiation using IR spectrophotometric technology, without the use of harsh chemicals and solvents.
- At lower levels of concentration, some of these concentration levels can “be directly detected by spectrometric analysis in the ultraviolet region (maximum absorbance at 260 nm) without any colour developer.”
- This new technology is preferred for its rapid, quantifiable, and non-destructive methods of analysis

Quality control :

- is another important factor in the analysis of fatty acids, especially for the processing and storage of edible oil products.
- Edible oils must undergo various stages of processing, which require constant monitoring of acid value ratios to ensure that the correct balance is maintained.
- When imbalances occur, it can result in a rancid smell and poor quality in both taste and appearance.
- Strict regulations apply to the processing and storage of such products, and many of these procedures require the regular use of colorimetric analysis through spectrophotometric technology.

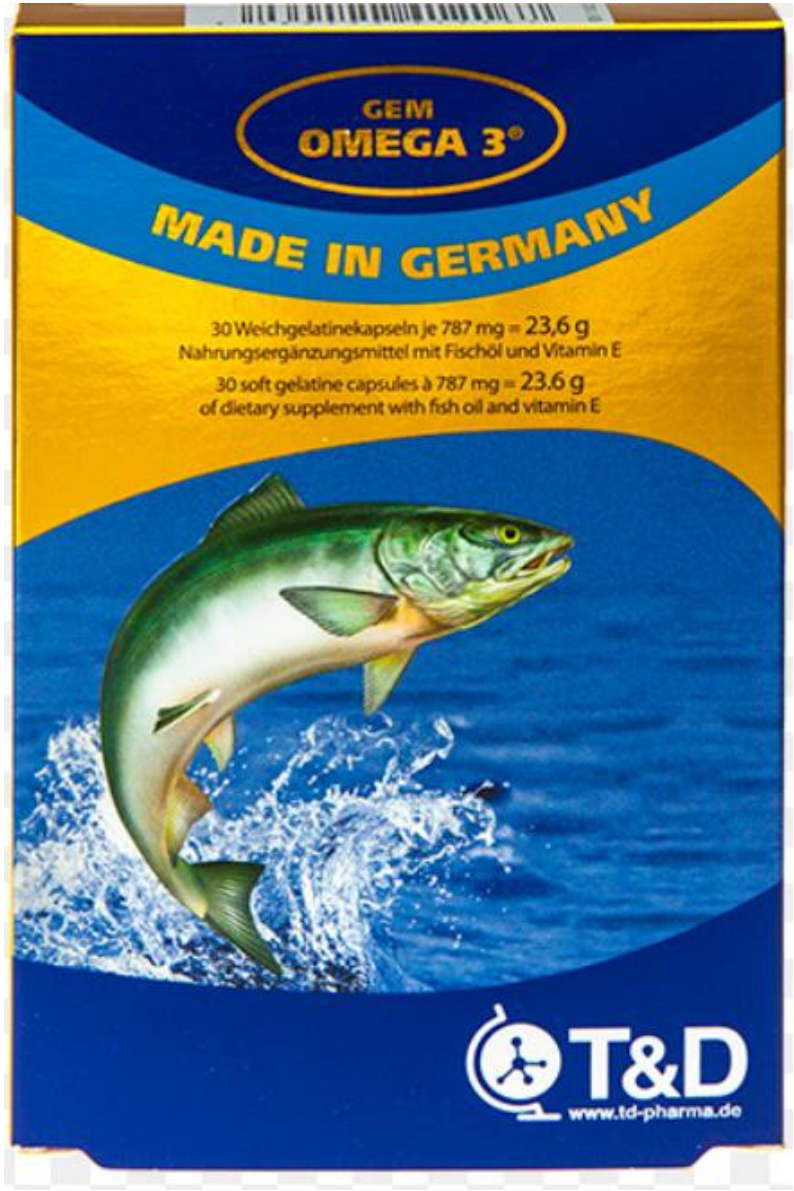
Colorimetric analysis instrumentation :

- **Spectrophotometry** is a widely-used and highly-developed method for color analysis, and is used for a variety of industrial applications.
- For use in the determination of fatty acids, spectrophotometers provide a non-invasive and safe alternative when compared to other methods of analysis.
- Available in a variety of design features and sizes, instrumentation options can fulfill specific application needs according to product structure and usage.

Analysis :

- The oils were obtained from soft-gel capsules of evening primrose oil, flax seed oil, black currant oil, and borage oil.
- The fats were initially in the form of triglycerides. They were saponified into their free acids and esterified for better volatility and inertness by GC.

The products that we analyse in our project :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Omega3
Plus Vitamin E
Source of
Omega3 fatty acid
(EPA and DHA)
30 SOFTGELS
1000 mg


Fish Oil
3 1
Omega 3
500
For a Healthy Heart
√ Supports Healthy Brain Function
√ Helps maintain Normal Vision
√ A Rich source of EPA&DHA
Food Supplement
30

Aim of the project :

- The aim of our project is to make analysis to omega 3 supplement capsules in the market to ensure if the product contain the active substance by using UV measurement Through spectrophotometer.
- We can conclude that if we see the curve of absorption (double bonds conjugation which indicate the unsaturation of the fat in the supplement and compare the absorbance to the absorbance of the reference of omega 3 the analysis is occur via different electronic transitions within the spectrophotometer.

Materials and tools :

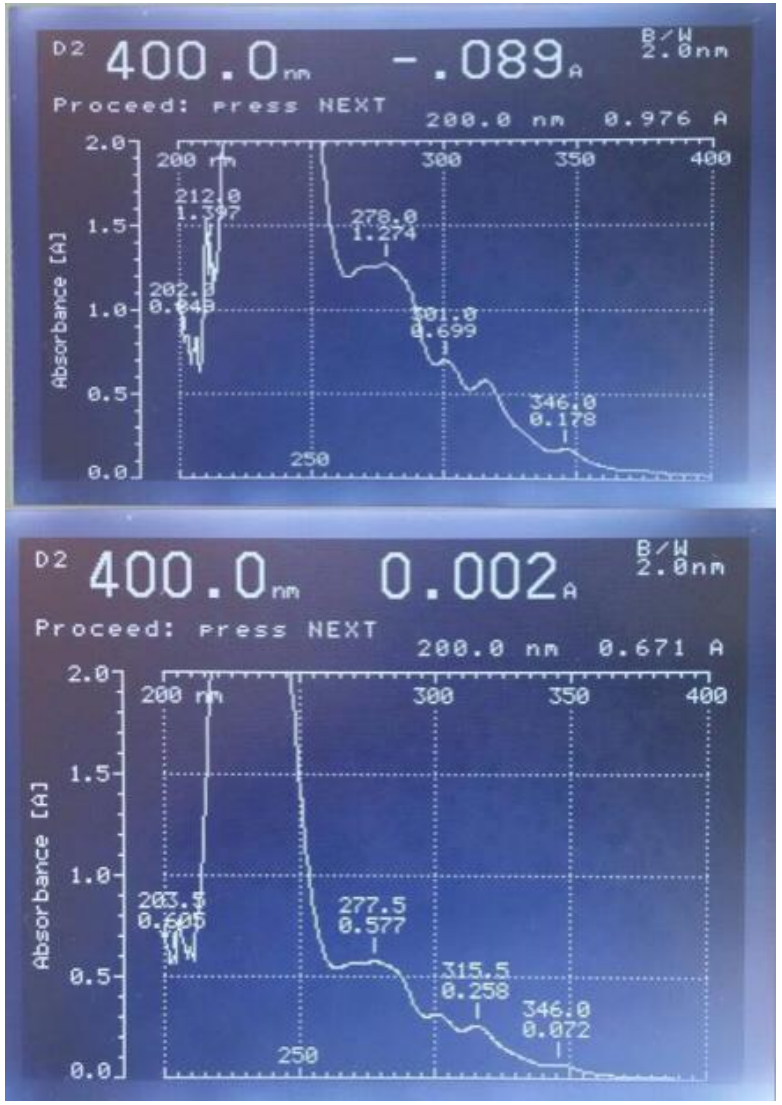
- **We use in our project :**
  - sofgel capsule of omega three from each company from the companies we use.
  - Unshell the capsule
  - Mix with 15 ml of ethanol)
  - Take 2ml from the mixture
  - Dilute with 2 ml of ethanol
  - Use spectrophotometer cells (one cell is filled with ethanol) and the other cell is filled with sample (to remove the absorbance of solvent so prevent scattering in UV measurement)

- Remake this procedure with each company product (we use 5 products in our project.
- In UV spectroscopy, the sample is irradiated with the broad spectrum of the UV radiation.
- If a particular electronic transition matches the energy of a certain band of UV, it will be absorbed.
- The remaining UV light passes through the sample and is observed From this residual radiation a spectrum is obtained.

Result :

- There are three electronic transitions(conjugation in the curve) in following two compounds curvesat wavelengths of (278 , 301 , 346)nm for the first one and (277,315,346)nm for the second one.
- So this range of wavelengths indicates n → p* and p → p* transitions which is for unsaturated and carbonyl containing compounds
- Most absorption spectroscopy of organic compounds is based on transitions of n or p electrons to the p* excited state.

- This is because the absorption peaks for these transitions fall in an experimentally convenient region of the spectrum (200 - 700 nm).
- These transitions need an unsaturated group in the molecule to provide the p electrons.


D2 400.0nm -.089A
B/W 2.0nm
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200.0 nm 0.976 A
Absorbance [A]
2.0
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1.0
0.5
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200 nm
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350
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212.0
1.397
278.0
1.274
202.
0.648
301.0
0.699
346.0
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D2 400.0nm 0.002A
B/W 2.0nm
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Absorbance [A]
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200 nm
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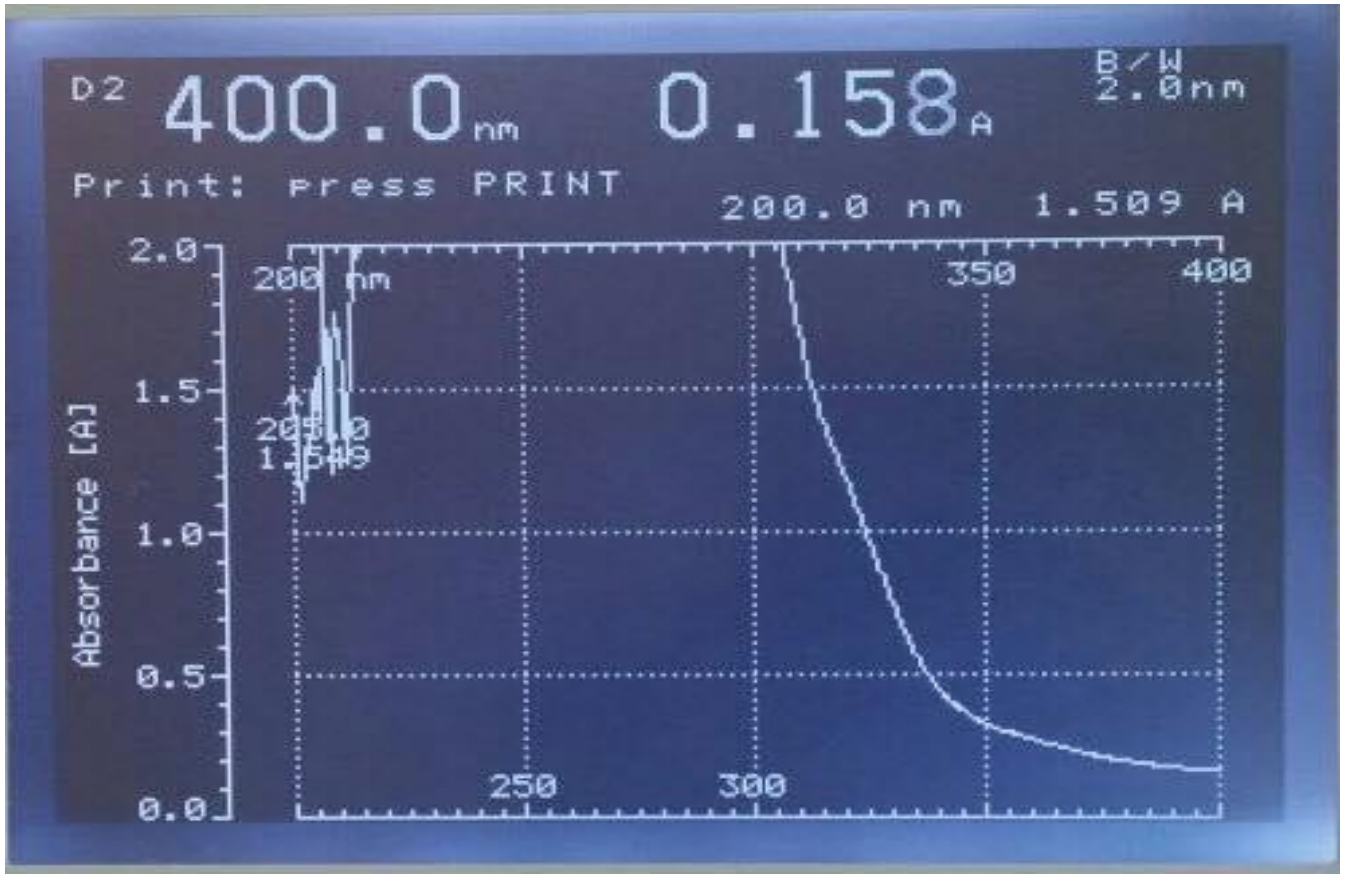
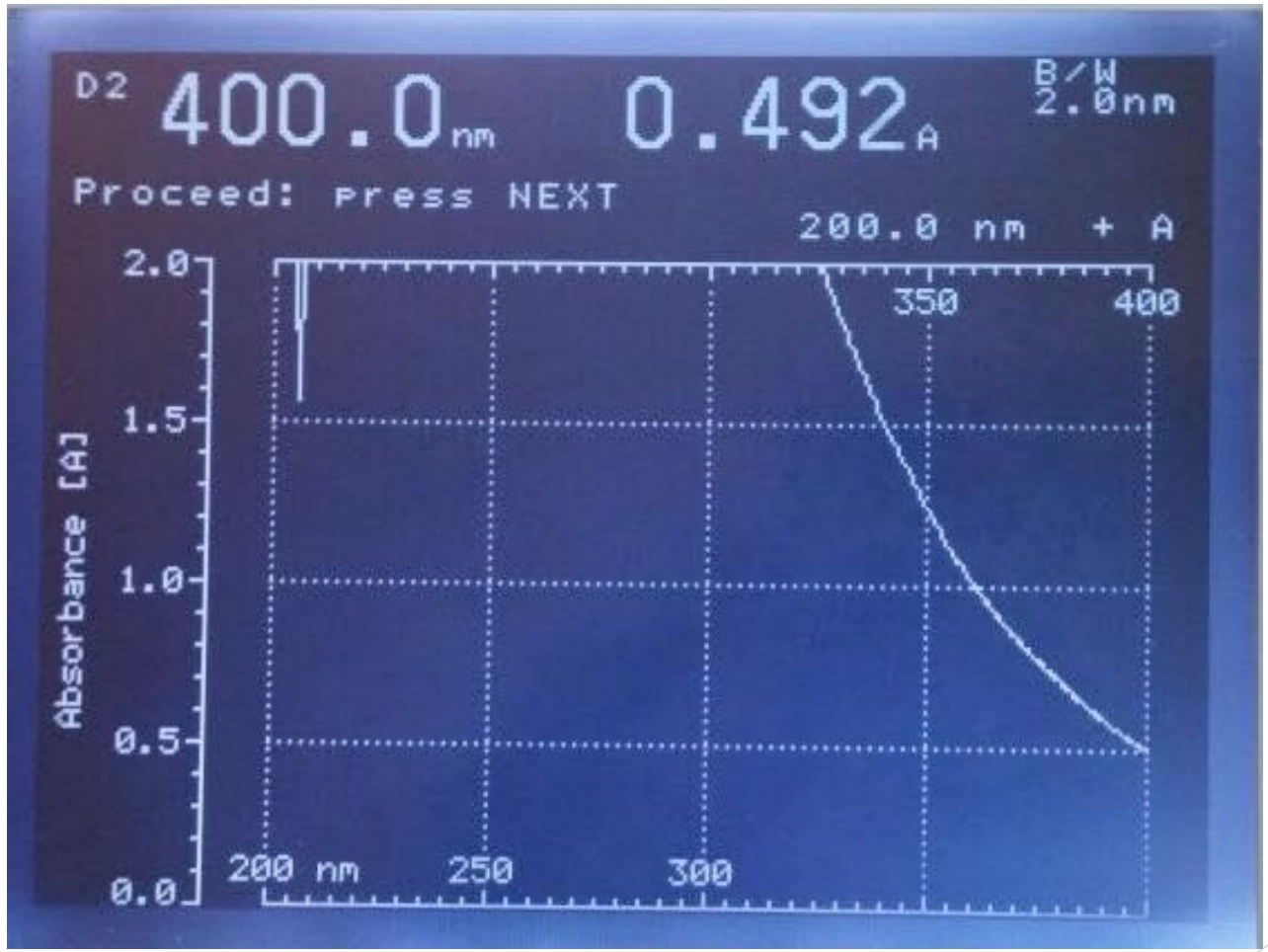
203.5
0.605
277.5
0.577
315.5
0.258
346.0
0.072
250

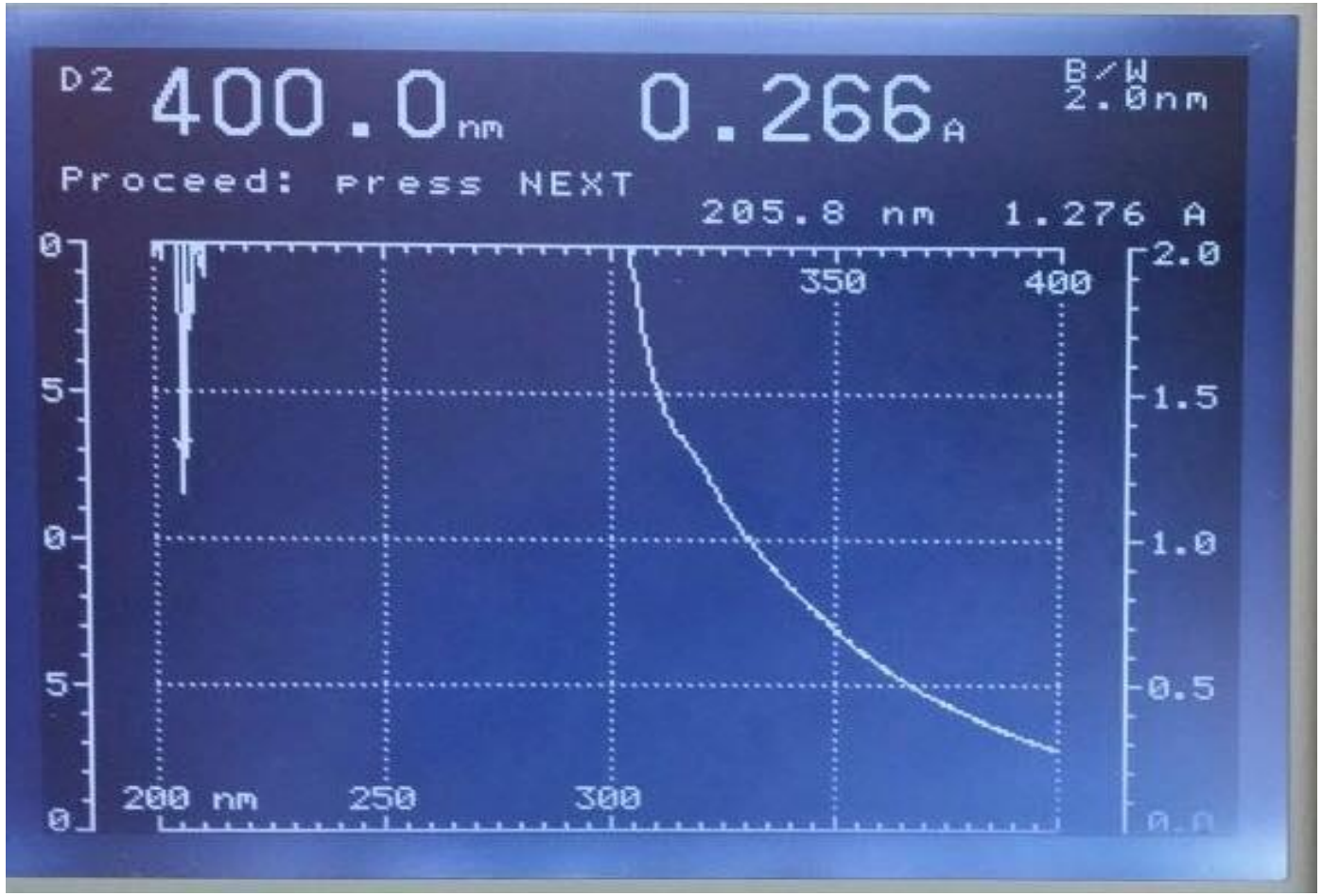
- This compound did not show any conjugation in Uv curve .



- This compound did not show any conjugation in Uv curve .



- This compound did not show any conjugation in UVcurve



Discussion :

- Only three of the five supplement show double bond unsaturation of fatty acid when tested by spectrophotometer ,Other supplement do not show any curve.

- Our test is considered only primary stage for the analysis of these supplement , So the drugs that not show curve may be due to lack of active substance or may be due to other causes **such as :**
  - chemical errors in our work .
  - Inaccuracy in work .
  - Errors in method of analysis (calculation difference) .
  - The solution may by concentrated due to other drugs and vitamins that found in combination with the product.
  - Excipients absorptivity may cause interference.

- Even the product that show double bonds in the UV curve so indicate unsaturated fatty acid but to ensure its omega 3 not other fatty acid need further analysis and detection methods but we did not make them because the time and availability of chemicals were limited factors

- It is important to mention that our project is not quality control subject for omega 3 and need more number of units that tested , its only simple analysis as in quality control there are several tests and techniques must be done to reach to the final result.
- Some of the products apperance was unacceptable after disolution the oil was turbid , sticky , near to be solid like so this also may be a cause of failure UV curve.
- So we can not reject or refuse the product upon our project because it is not quality control procedure.
- The solvent in which the absorbing species is dissolved also has an effect on the spectrum of the species.

- Peaks resulting from n to p* transitions are shifted to shorter wavelengths (blue shift) with increasing solvent polarity , This arises from increased solvation of the lone pair, which lowers the energy of the n orbital. Often (but not always), the reverse (i.e. red shift) is seen for p to p* transitions.
- This is caused by attractive polarization forces between the solvent and the absorber, which lower the energy levels of both the excited and unexcited states.

- This effect is greater for the excited state, and so the energy difference between the excited and unexcited states is slightly reduced - resulting in a small red shift.
- This effect also influences n to p* transitions but is over shadowed by the blue shift resulting from solvation of lone pairs.

Types of electronic transtions :

- σ → σ* :
  - High energy required, vacuum UV range
  - CH4: □ = 125 nm
- n → σ* :
  - Saturated compounds, CH3OH etc(□ = 150 - 250 nm)
- n → p* and p → p* :
  - Mostly used! □ = 200 - 700 nm

Reference :

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