# CHAPTER I: INTRODUCTION

Nicotine is the colourless or yellowish oily liquid which is the chief active constituent of tobacco. It acts as a stimulant in small doses, but in larger amounts blocks the action of autonomic nerve and skeletal muscle cells [13].

Nicotine is a dangerous and highly addictive chemical. It can cause an increase in blood pressure, heart rate, flow of blood to the heart and a narrowing of the arteries (vessels that carry blood). Nicotine may also contribute to the hardening of the arterial walls, which in turn, may lead to a heart attack. Nicotine is potentially harmful to non-users. At low amounts, it has a mild analgesic effect. The Surgeon General of the United States indicates that nicotine does not cause cancer. Nicotine has been shown to produce birth defects in some animal species, but not others. Nicotine has also been detected recently in spices, fresh herbs and herbal infusions, prompting the European Food Safety Authority to raise its maximum residue level in these foods to values between 0.03 and 4.0 mg/kg food [14].

Nicotine is used throughout the world for smoking, in cigarettes, cigars, pipes, etc., and is also chewed either alone or mixed with lime, etc. Nicotine is one of the most deadly poisons. Tobacco dry leaves contain from 0.6 to 6 percent of nicotine. Persons consuming excess of nicotine for a long period may suffer from chronic poisoning [12].

Nicotine is mainly administrated through ingestion and inhalation. Its fatal dose is 50mg. And the fatal period is uncertain minutes to hours. Burning pain, bitter taste, nausea, weakness, coma etc are the symptoms. Viscera, blood and urine are the evidentiary clues for the forensic examination. Paralysis of mid-brain and spinal cord are the action done by the poisonous substance “nicotine” [12].

Consuming nicotine through regular cigarettes or food leads to the release of the chemical dopamine in the human brain. As with many drugs, dopamine prompts or “teaches” the brain to repeat the same behaviour over and over. When nicotine is inhaled, the buzz you feel is the release of epinephrine which stimulates the body and causes your blood pressure and heart rate to increase, and

makes you breathe harder. Nicotine also activates a specific part of your brain that makes you feel happy by stimulating the release of the hormone dopamine [15].

Dark chocolate contains more caffeine than milk chocolate due to the higher proportion of cocoa solids that go into the product. The presence of other natural alkaloids in chocolate like nicotine and myosmine, a minor tobacco alkaloid, is more uncertain, with conflicting reports. In a way, nicotine chokes out tissues in the mouth from the blood it needs to survive, causing the death of gum tissues. Not enough saliva leads to bacteria build-up, dry mouth, and tooth decay. Nicotine acts as stimulant that fire up the muscles. If you already grind your teeth (bruise), it can make it worse [17].

At higher doses, however, nicotine enhances the effect of serotonin and opiate activity, exerting a calming and depressing effect. Nicotine-induced stimulation of the sympathetic nervous system leads to increased heart rate and blood pressure, cardiac stroke volume and output and coronary blood flow. Nicotine can be quantified in blood, plasma, or urine to confirm a diagnosis of poisoning or to facilitate a medico legal death investigation. Urinary or salivary cotinine concentrations are frequently measured for the purposes of pre-employment and health insurance medical screening programs. Careful interpretation of results is important, since passive exposure to cigarette smoke can result in significant accumulation of nicotine, followed by the appearance of its metabolites in various body fluids. Nicotine use is not regulated in competitive sports programs [15].

It will helpful for the identification for poisoning death. Nicotine, while highly addictive, is not a significant health hazard for people without heart conditions. It does not cause acute cardiac events, or the coronary heart disease, and is not carcinogenic. But nicotine is a problem for people with heart disease. Nicotine poisoning describes the symptoms of the toxic effects of nicotine following ingestion, inhalation, or skin contact. Nicotine poisoning can potentially be deadly, though serious or fatal overdoses are rare. The estimated lower limit of a lethal dose of nicotine has been

reported as between 500 and 1000 mg**.** Nicotine stops new brain cells forming. Nicotine can kill brain cells and stop new ones forming in the hippocampus, a brain region involved in memory. The French team finding explained the cognitive problems experienced by many heavy smokers during withdrawal, they say. Cell death also increased [(15).]

Ultraviolet visible spectroscopy or ultra violet visible spectrophotometer refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full, adjacent visible spectral regions. This means it uses light in the visible and adjacent [near ultraviolet and infrared (NIR)] range. Ultraviolet-Visible Spectrophotometer is used in the quantitative determination of concentration of the solutions [18].

Ultraviolet Visible spectroscopy is routinely used in analytical chemistry for the quantitative determination of different analytes, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules. Solvent polarity and pH can affect the absorption spectrum of an organic compound [18].

Principle of UV Spectroscopy, it obeys the Beer-Lamberts Law, which states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, Ultraviolet Visible spectroscopy can be used to determine the concentration of the absorber in a solution. The absorbance changes with concentration. This can be taken from reference [18].

One of the two divided beams is passed through the sample solution and second beam is pass through the reference solution are contained in the cells. The cells are made of either silica or quartz. Glass can't be used for the cells as it also absorbs light in the UV region [18].

Applications of Ultraviolet Visible Spectroscopy are it is used in Forensic Chemistry, Toxicology, Explosives, Narcotic drug analysis, Serology, DNA Fingerprinting etc. for qualitative and quantitative analysis of unknown samples [18].

Standard textbooks, databases and safety sheets consistently state that the lethal dose for adults is 60mg or less (30-60mg), leading to safety warnings that ingestion five cigarettes or 10ml of a dilute nicotine containing solution could kill an adult. Two hours after ingesting nicotine, the body will have removed around half of the nicotine. This means that nicotine has a half-life of around 2 hours. This short half-life means that the immediate effects of nicotine go away quickly, so people soon feel like they need another dose. Researchers have frequently indicated that the lethal dose of nicotine for adults is 50 to 60 milligrams (mg), which prompted safety warnings stating that approximately five cigarettes or 10 millilitres (ml) of a nicotine-containing solution could be fatal [14].

Nicotine also naturally occurs in smaller amounts (varying from 2-7 µg/kg, or 20-70 millionths of a percent wet weight in solanaceaein plants from the family solanaceae. Such as potatoes, tomatoes, eggplant and peppers [2]

The present study is aim to Detection of Nicotine in Various Chocolates. In past it has been found that there is only two study available related to Detection of Nicotine in Various Chocolates. In both the studies they were used solid-phase micro extraction coupled with Gas chromatography-tandem mass spectrometry. Melted portions of chocolates were mixed with HCL and centrifuged, before aqueous layer was mixed with sodium hydroxide solution followed by solid potassium carbonate for phase transfer. The alkaloids solution was extracted from the headspace volume by solid-phase micro extraction then the fibre was removed and inserted into the inlet of the GC. It was heated to desorb the compounds which were separated on the column for electron ionisation and multiple reaction monitoring [19].

In the present study melted portion of the chocolates where treated with distilled water and by using filter paper the filtration of solution is done. The samples were taken in the cuvettes for the further examination in UV spectroscopy by using Halogen Lamp.

# CHAPTER II: LITERATURE REVIEW

Neil Gruenberg et.al (1985) identified the importance of sweet taste and caloric content in the effects of Nicotine on specific food consumption. They found that there is an inverse relationship between nicotine and body weight that has been partially explained by changes in consumption of sweet-tasting high calorie foods. Body weight, food consumption and water consumption were measured daily before, during, and after during consumption. In all three experiments, there was an inverse relationship. These findings have implications for controlling body weight gains after cessation of cigarette smoking.

B.E Schroeder et.al (2001) identified a common profile of prefrontal cortical activation following exposure to nicotine or chocolate associated contextual cues. Conditioning and learning factors are likely to play key roles in the process of addiction and in relapse to drug use. Using the detection of the immediate early gene products, FOS, they examined which regions of the brain are activated by environmental cues associated with nicotine administration, and compared this profile to the pattern induced by cues associated with the natural reward, chocolate, induced a pattern of gene expression that showed many similarities with that elicited by drug cues, particularly in prefrontal regions.

Jose Antonio Baz-Lomba et.al (2016) identified the comparison of pharmaceutical illicit drug, alcohol, nicotine and caffeine levels in waste water with sale, seizure and composition data. Waste water based epidemiology has been shown to be a reliable approach complementing. The study aims to compare and correlate the consumption estimates of pharmaceuticals, illicit drugs, alcohols, nicotine and caffeine from waste water analysis and other sources of information. Waste water collected from eight different European cities over a one week period, representing a population of approximately 5 million people. This work confirms the promising future of WBE as a complementary approach to obtain a more accurate picture of substance use situation within different communities.

Antonella Aresta et.al (2004) identified Simultaneous determination of caffeine, theobromine, theophylline, paraxanthine and nicotine in human milk by liquid chromatography with diode array UV detection. The method has been applied to human milk samples. The within-day ($n$ = 5) and between-days ($n$ = 5 over 5 days) coefficients of variation in milk ranged from 3.7% (caffeine) to 4.7% (theobromine) and from 5.1% (caffeine) to 6.7% (theobromine). Estimated LOD and LOQ in milk ranged from 8 (caffeine) to 13 (nicotine) ng/ml and from 24 (caffeine) to 34 (nicotine) ng/ml, respectively.

Gideon St. Helen et.al (2017) identified Impact of e-liquid flavours on nicotine intake and pharmacology of e-cigarettes. Rate of nicotine absorption was different between strawberry and tobacco e-liquids. The pH of e-liquids may influence rate of nicotine absorption. Vapours titrate their nicotine exposure but extent of titration varies with flavour. Flavours influence nicotine exposure through flavour liking, may affect rate of nicotine absorption possibly through pH effects, and contribute to heart rate acceleration and subjective effects of e-cigarettes. E-cigarette users titrate their nicotine exposure but the extent of titration may vary across flavours.

Shuh J. Sheen et.al (September 1988) determined the detection of nicotine in foods and plants materials. Nicotine at several ppm was detected in the dehydrated fresh produce of the Solanaceae species including tomato, potato peel, eggplant and green pepper. Its identity was verified by GLC, TLC and CC-mass spectrometry. The presence of nicotine in all parts of the tomato plant suggested biosynthetic origin. In contrast, the 2 to 23 ppm nicotine found in green tea and instant tea samples might be attributed to insecticide contamination. There was no detectable level of nicotine in non-Solanaceae fruit and vegetables and other processed foods analyzed.

Paola A. Magni et.al (2015) identified the development and validation of a GC-MS method for nicotine detection in callipora vomitoria (L.) [Diptera: Calliphoridae]. Entomotoxicology is the application of toxicological methods and analytical procedures on necrophagous insects feeding on decomposing tissues to

detect drugs and other chemical components, and their mechanisms affecting insect development and morphology and modifying the methodology for estimation of minimum time since death. Nicotine is a readily available potent poison. Because of its criminal use, a gas chromatography–mass spectrometry (GC–MS) method for the detection of nicotine in Calliphora vomitoria L. (Diptera: Calliphoridae) was developed and validated. Furthermore, the effect of nicotine on the development, growth rate, and survival of this blowfly was studied. Larvae were reared on liver substrates homogeneously spiked with measured amounts of nicotine (2, 4, and 6 ng/mg) based on concentrations that are lethal to humans. The results demonstrated that (a) the GC–MS method can detect both nicotine and its metabolite cotinine in immature *C.* vomitoria*;* (b) the presence of nicotine in the aforementioned three concentrations in food substrates did not modify the developmental time of *C.* vomitoria*;* (c) during the pupation period, larvae exposed to nicotine died depending on the concentration of nicotine in the substrate; and (d) the resultant lengths of larvae and pupae exposed to 4 and 6 ng/mg concentrations of nicotine were significantly shorter than those of the control [(9).]

Tania S. Agostini et.al (April 2005) identified the simultaneous determination of nicotinamide, nicotinic acid, riboflavin, thiamin, and pyridoxine in enriched Brazilian foods by HPLC. The control of the enrichment levels in foods is difficult, due mainly to the lack of appropriate analytical methodologies. The amounts of the five B-group vitamins (nicotinamide, nicotinic acid, riboflavin, thiamin, and pyridoxine) have been determined in enriched Brazilian foods by a high-performance liquid chromatography (HPLC) method. Fifty products, such as biscuits, liquid and dry milks, flavoured milk drinks, flour, macaroni, and cereals were analyzed. Some products showed the amounts declared on the package. Although some slight quantitative variations were shown in the biscuits, one showed levels of riboflavin 35% lower than the value declared. Of five different corn cereal brands, only one showed the declared vitamin content, the others showing levels 30 % lower than that declared. No B-group vitamins were detected in one brand of enriched macaroni, except for the nicotinic acid naturally present in the flour. On the other hand, one flavoured milk

drink exhibited vitamins levels 200% higher than the amounts declared and one milk drink mix presented thiamin, riboflavin, and nicotinamide levels 3 to 5 times greater than stated. These results suggest an absence of control of the amount of vitamins in enriched foods.

M. J. Martinez Bueno et.al (2011) determined the evolution of selected ubiquitous contaminants in the aquatic environment and their transformation products. A pilot study of their removal from a sewage treatment plant. A simple method using direct sample injection combined with liquid chromatography tandem mass spectrometry has been developed for the simultaneous analysis of six alkaloid compounds in environmental samples. The target list includes two psycho stimulants (nicotine and caffeine), three metabolites (cotinine, nicotinic acid and paraxanthine) and a coffee chemical (trigonelline). The analytical method was evaluated in three different matrices (surface water, influent and effluent wastewater). The method developed showed an adequate sensitivity, below 0.6 $\mu g\ L^{-1}$ for wastewater and 0.1 $\mu g\ L^{-1}$ for river matrices, without any prior treatment of the samples. Finally, the methodology was applied to real samples for evaluation of their removal from a sewage treatment plant and their persistence/fate in the aquatic environment. All compounds studied in this work were detected at all sampling points collected along the Henares River. However, nicotinic acid was only detected three times in treated sewage samples at levels above its detection limit.

Manuela Pellegrini et.al (20 July 2007) identified the liquid chromatography/electro spray ionization tandem mass spectrometry assay for determination of nicotine and metabolites, caffeine and arecoline in breast milk. A procedure based on liquid chromatography/tandem mass spectrometry (LC/MS/MS) is described for the determination of nicotine and its principal metabolites cotinine, trans-3-hydroxycotinine and cotinine-N-oxide, caffeine and arecoline in breast milk, using N-ethylnorcotinine as internal standard. Liquid/liquid extraction with chloroform/isopropanol (95:5, v/v) was used for nicotine, cotinine, trans-3-hydroxycotinine, cotinine-N-oxide and caffeine under neutral conditions and for arecoline under basic conditions. Chromatography was

performed on a $C_8$ reversed-phase column using a gradient of 50 mM ammonium formate, pH 5.0, and acetonitrile as a mobile phase at a flow rate of 0.5 mL/min. Separated analytes were determined by electro spray ionization tandem mass spectrometry in the positive ion mode using multiple reaction monitoring. Limits of quantification were 5 µg/L for nicotine, cotinine, trans-3-hydroxycotinine, cotinine-N-oxide and caffeine, and 50 µg/L for arecoline using 1 mL human milk per assay. Calibration curves were linear over the calibration ranges for all the substances under investigation, with a minimum $r^2 > 0.998$. At three concentrations spanning the linear dynamic range of the assay, mean recoveries from breast milk ranged between 71.8 and 77.4% for different analytes. This method was applied to the analysis of analytes in human milk to assess substance exposure in breast-fed infants in relation to eventual clinical outcomes. This LC/MS/MS assay provides adequate sensitivity and performance characteristics for the simultaneous quantification of biomarkers of three of the drugs most commonly used worldwide (tobacco, caffeine and areca nut).

Peter M. Clayton et.al (2013) identified the Spectroscopic Studies on Nicotine and Nor-nicotine in the UV Region. The UV absorption and electronic circular dichroism (ECD) spectra of (R) - and (S) -nicotine and (S) -nor-nicotine in aqueous solution were measured to a significantly lower wavelength range than previously reported, allowing the identification of four previously unobserved electronic transitions. The ECD spectra of the two enantiomers of nicotine were equal in magnitude and opposite in sign, while the UV absorption spectra were coincidental. In line with previous observations, (S) -nicotine exhibited a negative cotton effect centred on 263 nm with vibronic structure ($\pi$–$\pi_1$* transition) and a broad, positive ECD signal at around 240 nm associated with the n–$\pi_1$* transition. As expected this band disappeared when the pyridyl aromatic moiety was protonated. Four further electronic transitions are reported between 215 and 180 nm; it is proposed the negative maxima around 206 nm is either an n–$\sigma$* transition or a charge transfer band resulting from the movement of charge from the pyrrolidyl N lone pair to the pyridyl $\pi$* orbital. The pyridyl $\pi$–$\pi_2$* transition may be contained within the negative ECD signal envelope at around 200 nm. Another negative maximum at 188 nm is thought to be the pyridyl $\pi$–$\pi_3$*

transition, while the lowest wavelength end-absorption and positive ECD may be associated with the $\pi$–$\pi_4$* transition. The UV absorption spectra of (S)-nor-nicotine was similar to that of (S)-nicotine in the range 280–220 nm and acidification of the aqueous solution enhanced the absorption. The ECD signals of (S) -nor-nicotine were considerably less intense compared to (S) -nicotine and declined further on acidification; in the far UV region the ECD spectra diverge considerably.

## CHAPTER III: AIM and OBJECTIVES

## Aim

To detect the presence of nicotine in various brands of chocolates by using UV Spectroscopy

## Objectives

- To identify the range of nicotine in particular chocolates with the help of peaks.
- To know how the range will vary from one brand to another brand.

# CHAPTER IV: MATERIALS AND METHODOLOGY

## Materials Required:

### Apparatus:

1. conical flask
2. funnel
3. filter paper
4. forceps

### Reagent:

1. Distilled water
2. sample

### Instrument required:

1. Weighing machine. KEROY Company with a model KRT 1000.
2. Ultraviolet Visible spectroscopy. Analytik jena company with a model SPECORD 200 Plus.

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**Figure 1: Weighing Machine**


**Figure 2: UV Spectroscopy**

## Method:

Seven various brands of chocolates were collected. Wearied gloves before the sample collection. Piece of paper is taken and made it as a weighing plate. From each branded chocolate 1gm of sample is taken in the conical flask by using forceps. And add 50ml of distilled water. After diluting the sample in the solution. Filtrated the sample by using filter paper and in the conical flask. Due to the high density the filtration took time. Switched on the UV spectroscopy and started to measure the range of sample. The software used in the UV spectroscopy is Aspect UV.Before measuring each sample, measurement of reference sample is taken. Then evaluated the peaks of the samples in between 200nm to 300nm.Screenshot of the each sample is taken. Documented the peaks and find out the range of nicotine by reading the graphs.

**Figure 3: Sample**

**Figure 4: Filtration**

# CHAPTER V: OBSERVATIONS

## GALAXY

| Samples | Range (nm) | Absorption | Average |
|---|---|---|---|
| Sample 1 | 223 nm | 2.97 | 3.15 |
| Sample 2 | 232 nm | 3.14 | |
| Sample 3 | 229 nm | 3.24 | |
| Sample 4 | 227 nm | 3.22 | |
| Sample 5 | 229 nm | 3.18 | |

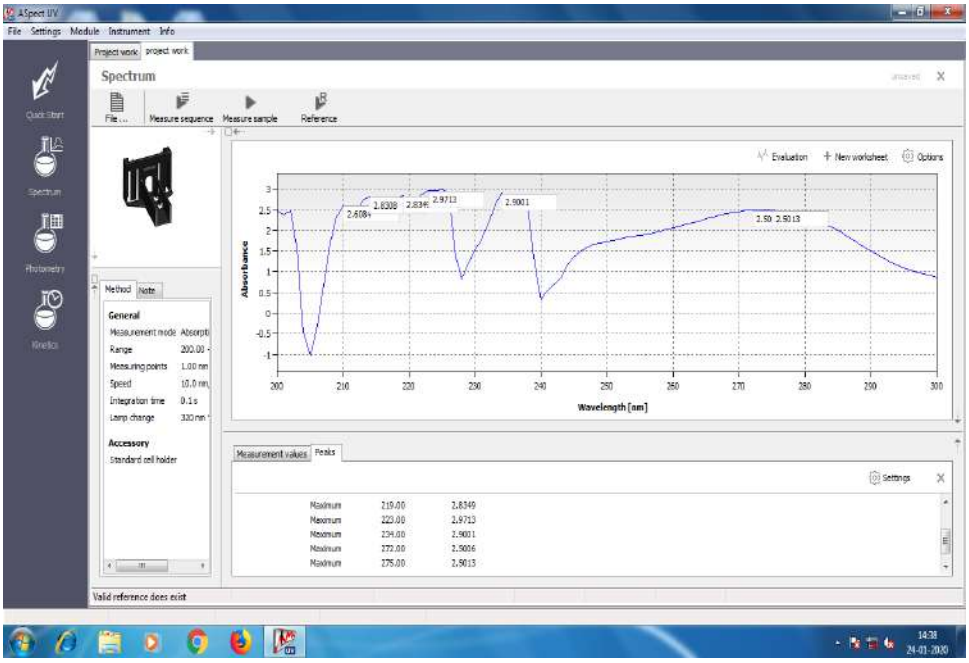


**Figure 5: UV graph of Galaxy Sample 1**

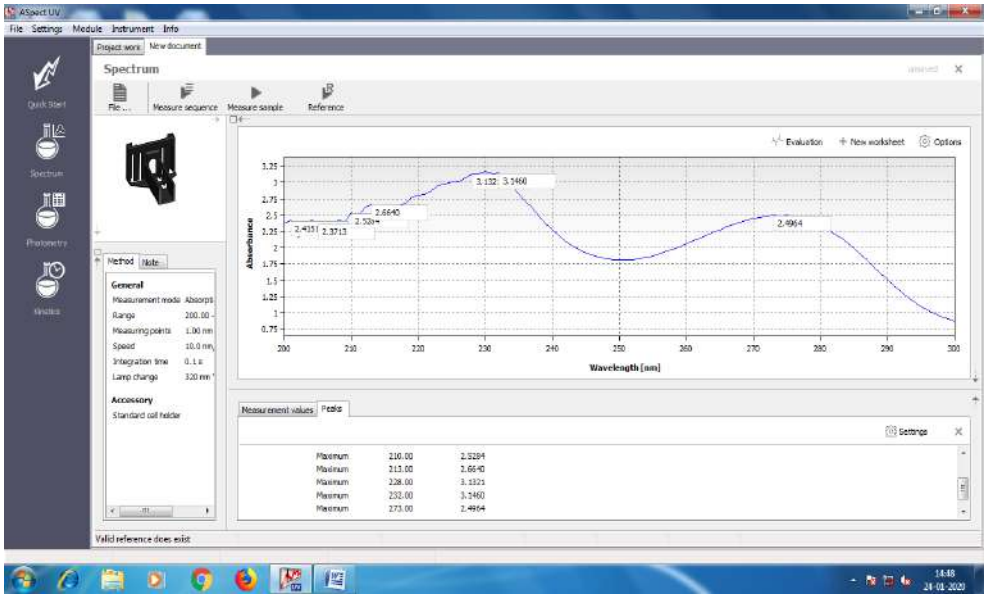


**Figure 6: UV graph of Galaxy Sample 2**

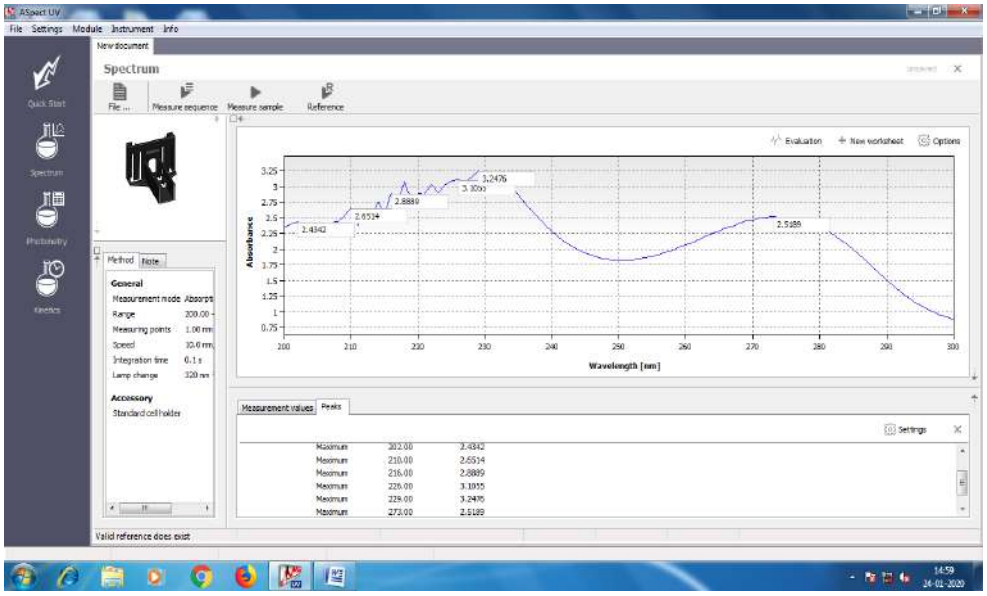


**Figure 7: UV graph of Galaxy Sample 3**

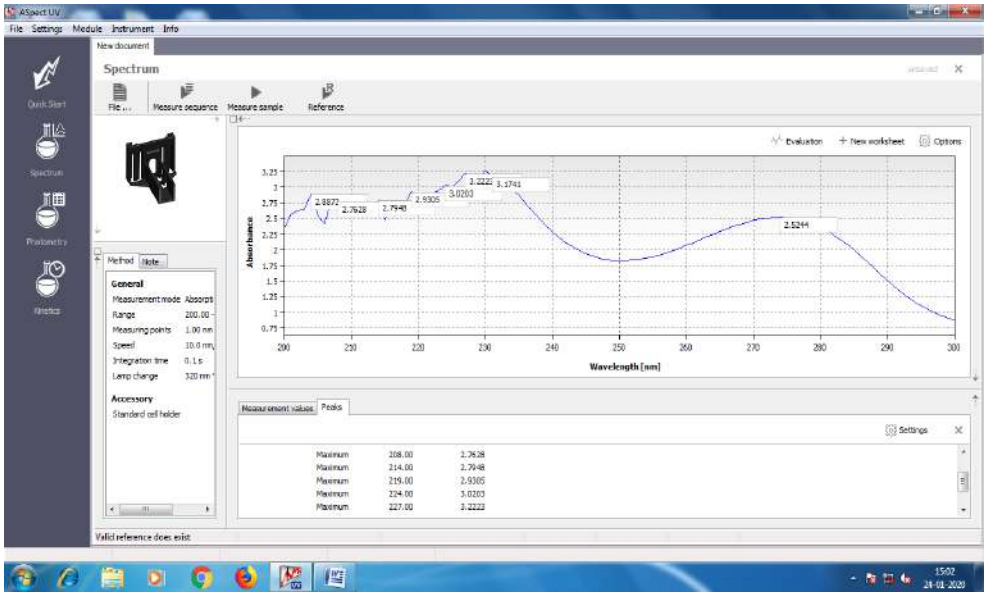


**Figure 8: UV graph of Galaxy Sample 4**

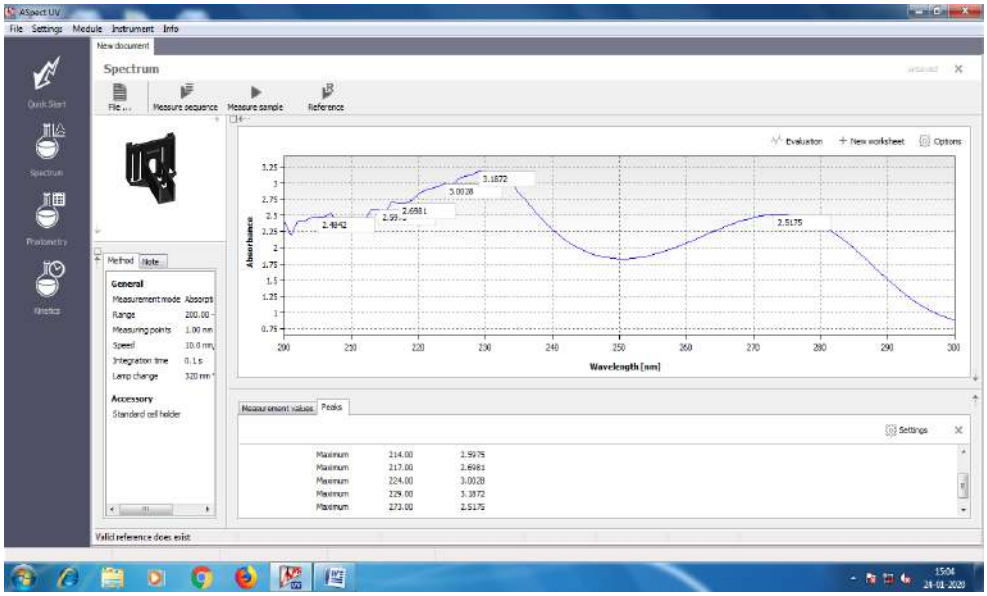


**Figure 9: UV graph of Galaxy Sample 5**

## TIFFANY ÉCLAIR

| Samples | Range(nm) | Absorption | Average |
|---|---|---|---|
| Sample 1 | 211 nm | 2.55 | 2.546 |
| Sample 2 | 208 nm | 2.48 | |
| Sample 3 | 205 nm | 2.59 | |
| Sample 4 | 211 nm | 2.59 | |
| Sample 5 | 212 nm | 2.52 | |

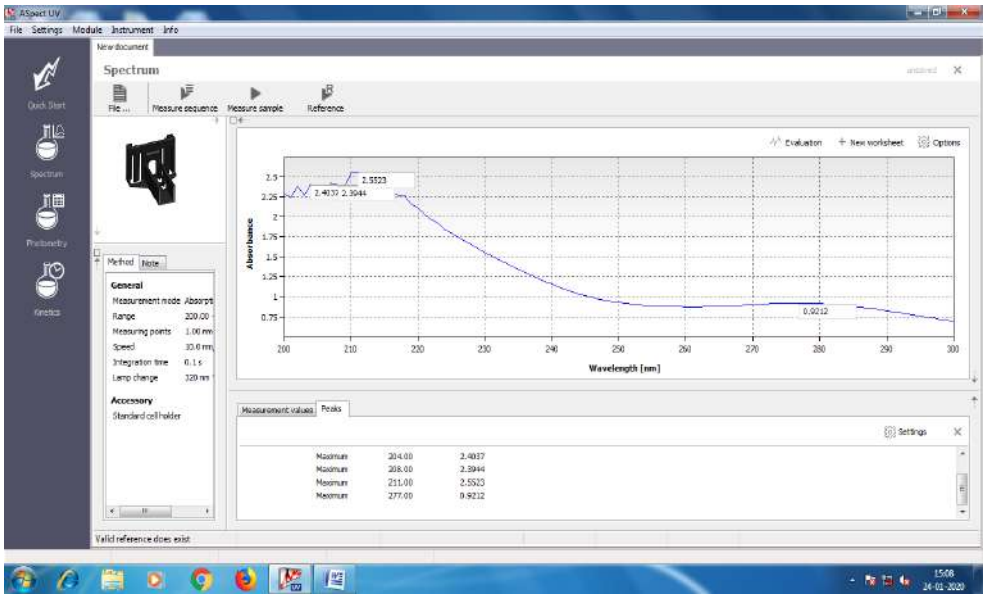


**Figure 10: UV graph of Tiffany Éclair Sample 1**

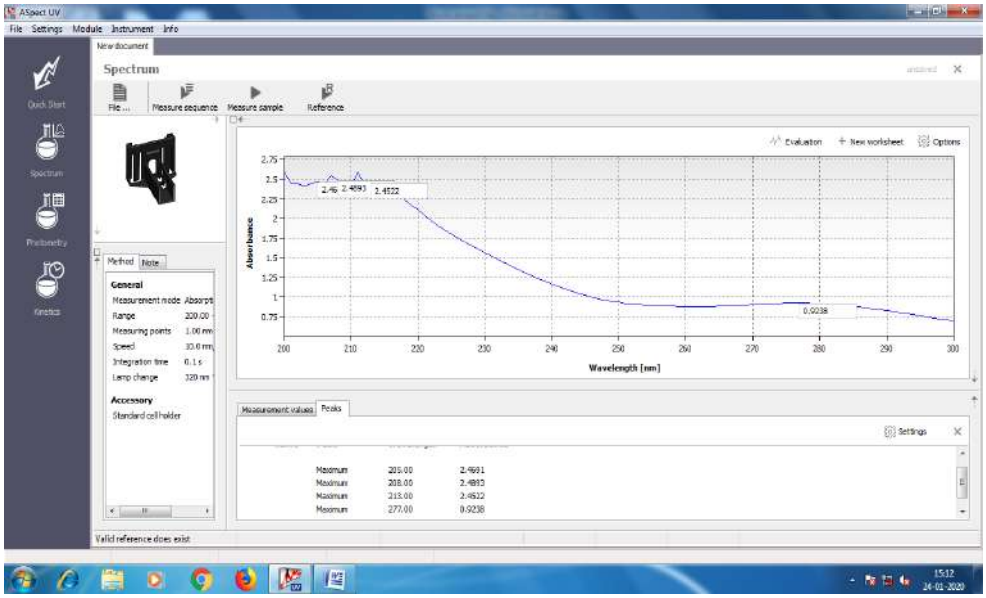


**Figure 11: UV graph of Tiffany Éclair Sample 2**

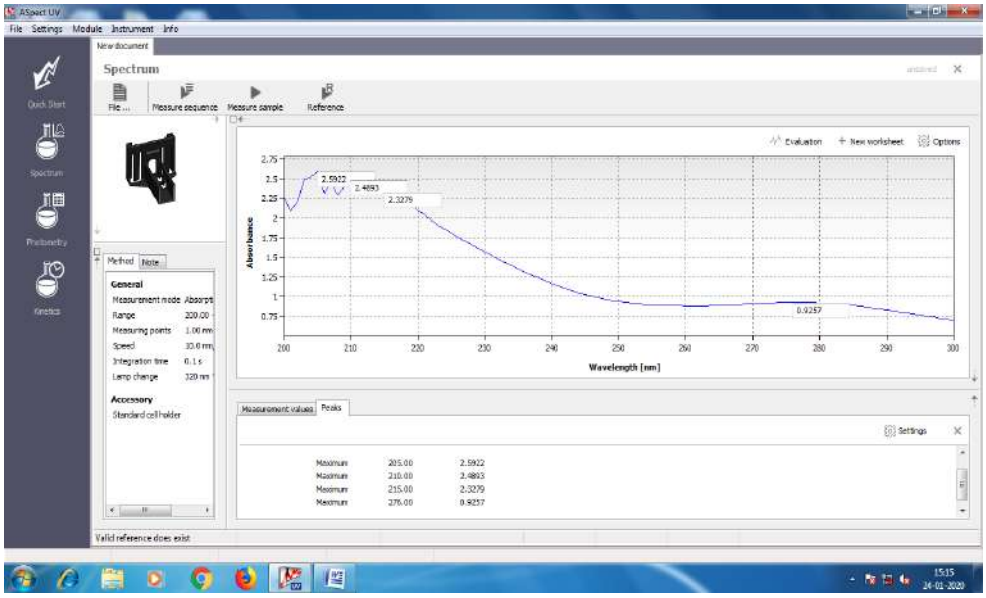


**Figure 12: UV graph of Tiffany Éclair Sample 3**

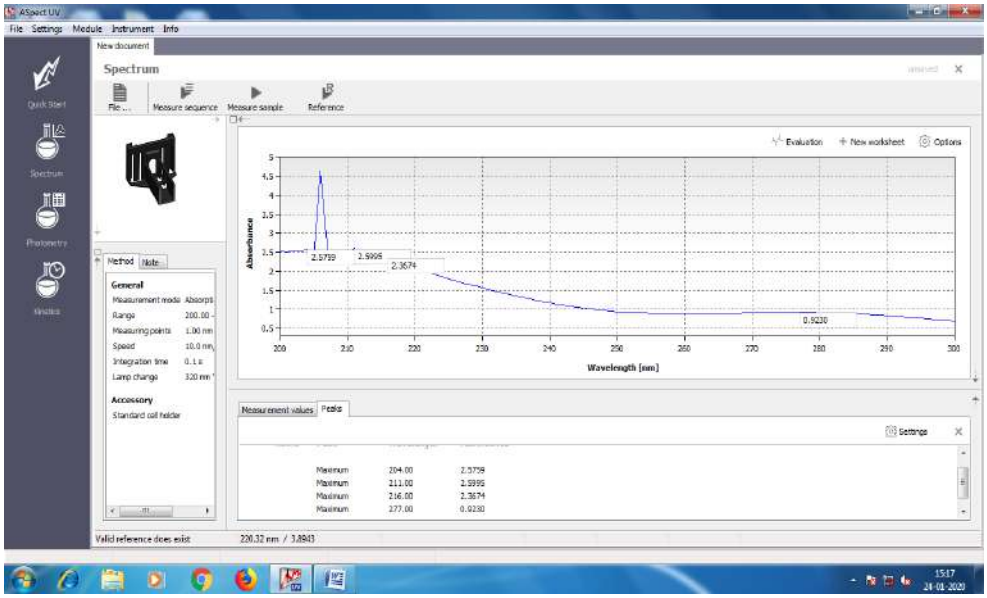


**Figure 13: UV graph of Tiffany Éclair Sample 4**

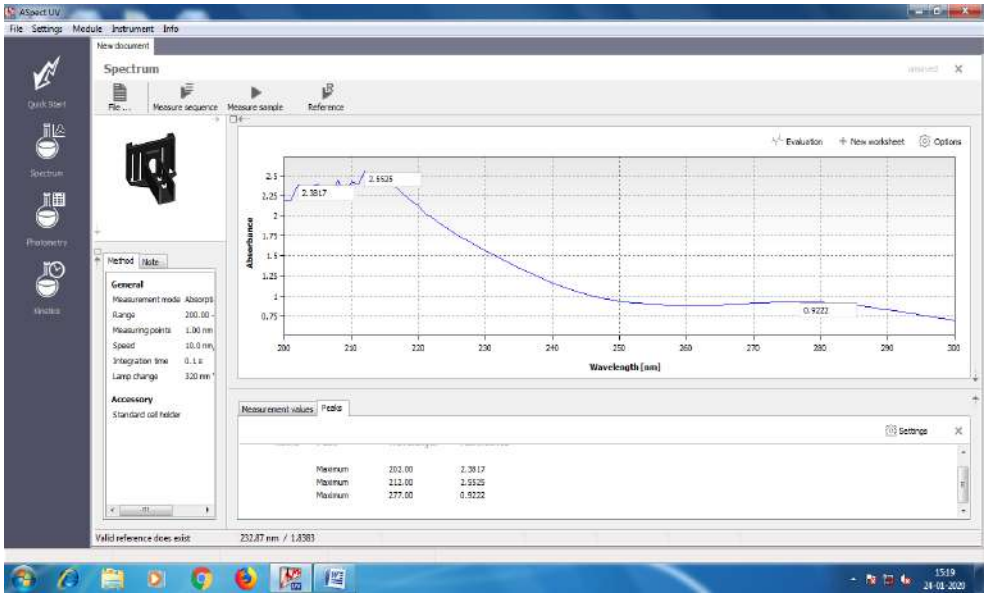


**Figure 14: UV graph of Tiffany Éclair Sample 5**

## CHOCOLALA

| Samples | Range (nm) | Absorption | Average |
|---|---|---|---|
| Sample 1 | 236 nm | 3.65 | 3.622 |
| Sample 2 | 237 nm | 3.57 | |
| Sample 3 | 237 nm | 3.61 | |
| Sample 4 | 274 nm | 3.61 | |
| Sample 5 | 239 nm | 3.67 | |

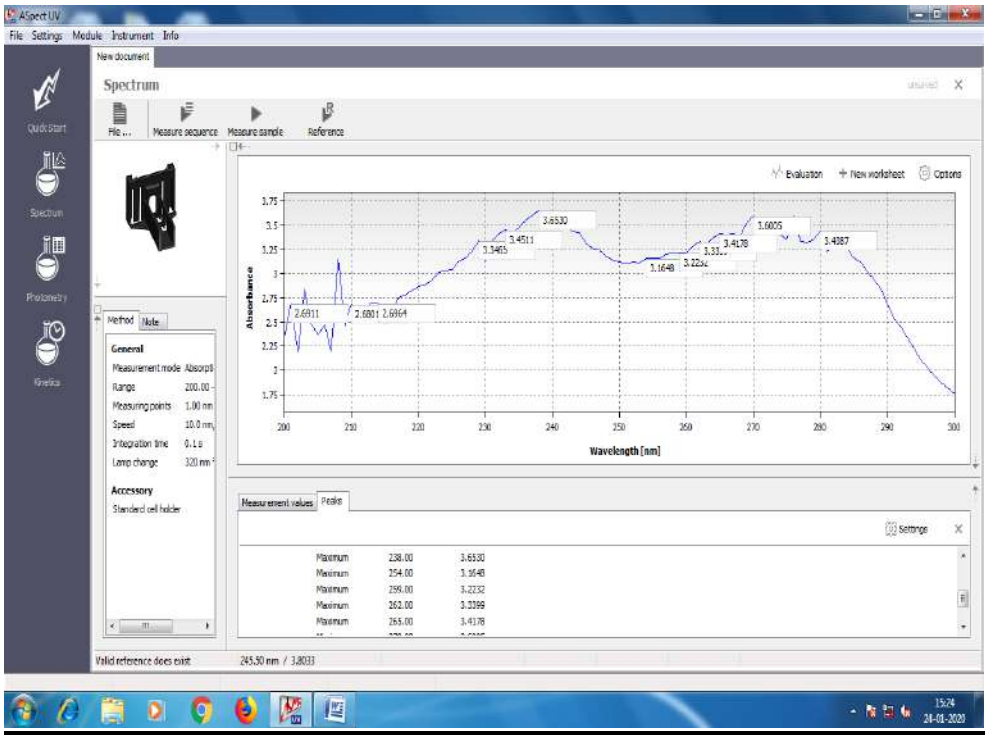


**Figure 15: UV graph of Chocolala sample 1**

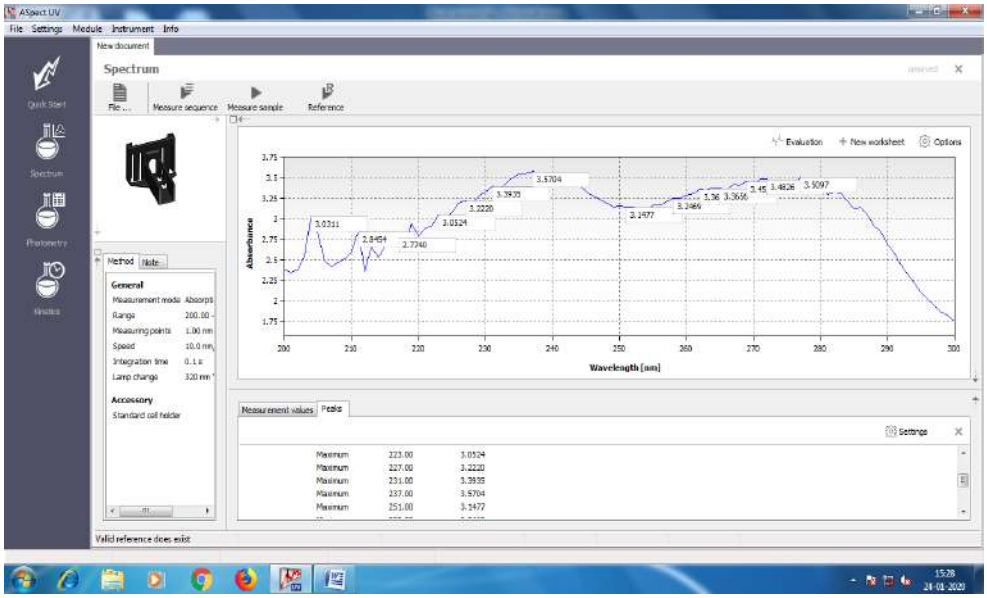


**Figure 16: UV graph of Chocolala sample 2**

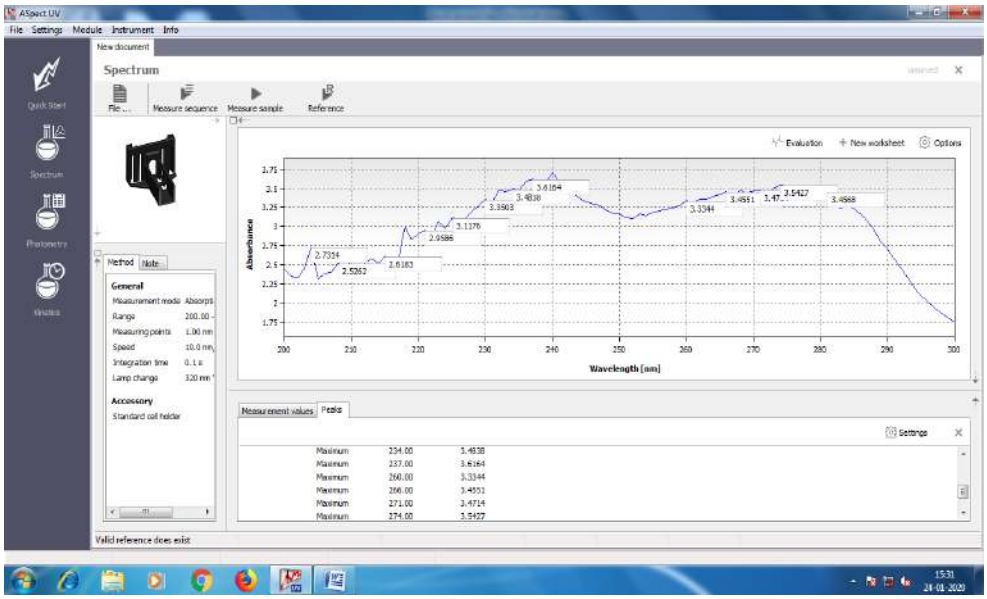


**Figure 17: UV graph of Chocolala sample 3**

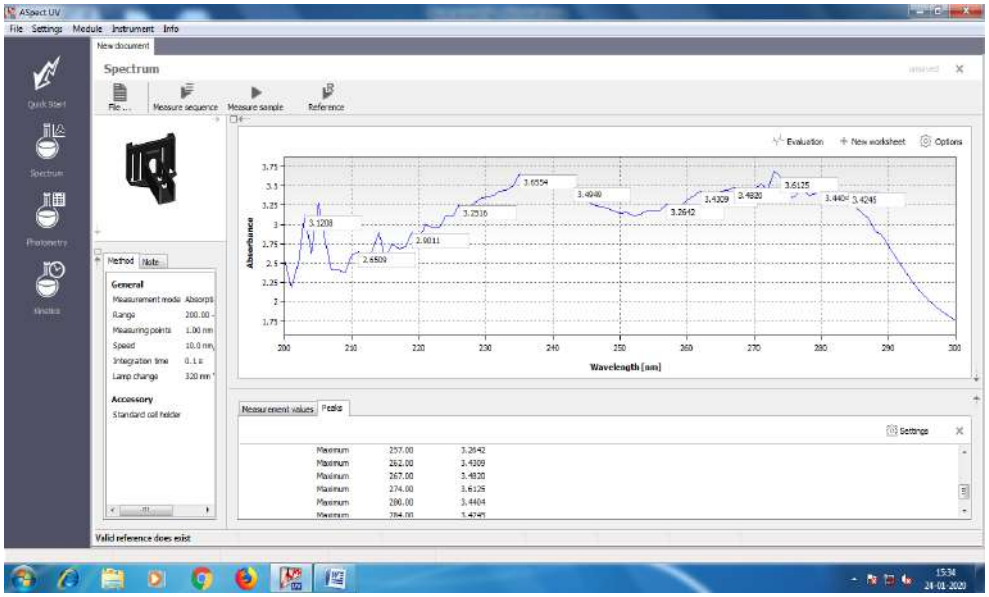


**Figure 18: UV graph of Chocolala sample 4**

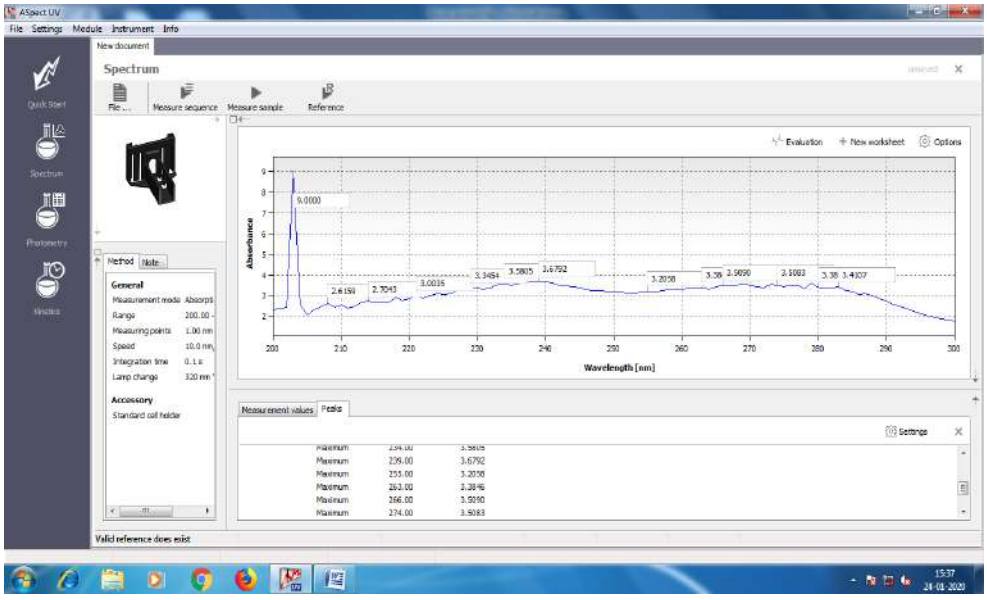


**Figure 19: UV graph of Chocolala sample 5**

## TIFFANY

| Samples | Range (nm) | Absorption | Average |
|---|---|---|---|
| Sample1 | 242 nm | 3.70 | 3.732 |
| Sample2 | 242 nm | 3.75 | |
| Sample3 | 238 nm | 3.79 | |
| Sample4 | 272 nm | 3.71 | |
| Sample5 | 245 nm | 3.71 | |

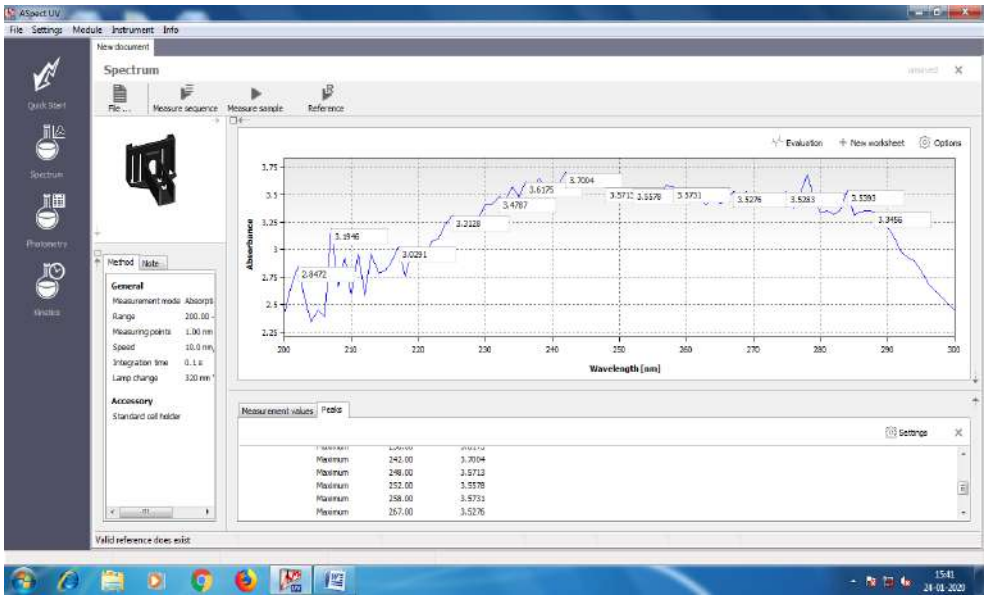


**Figure 20: UV graph of Tiffany Sample 1**

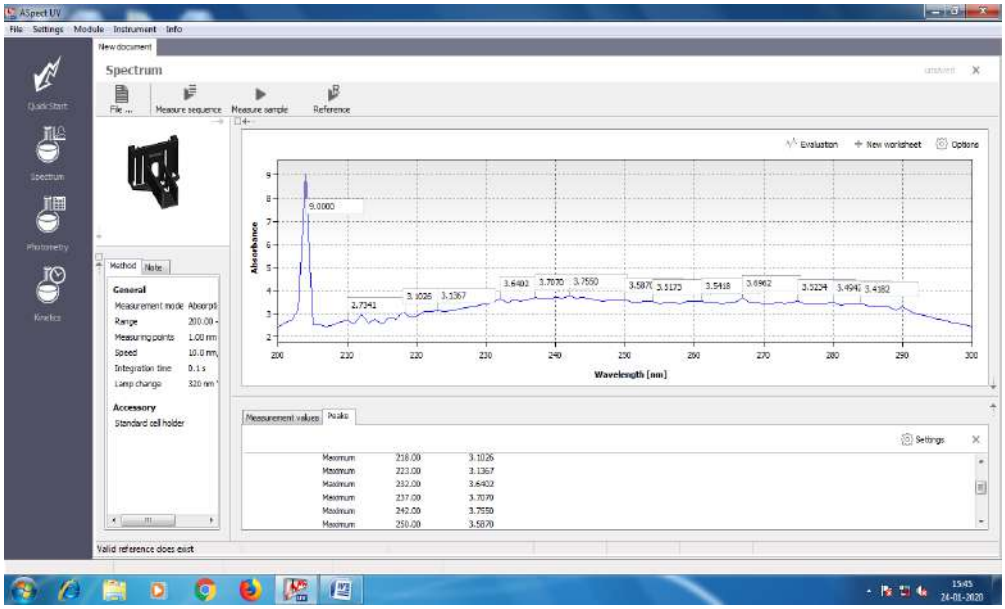


**Figure 21: UV graph of Tiffany Sample 2**

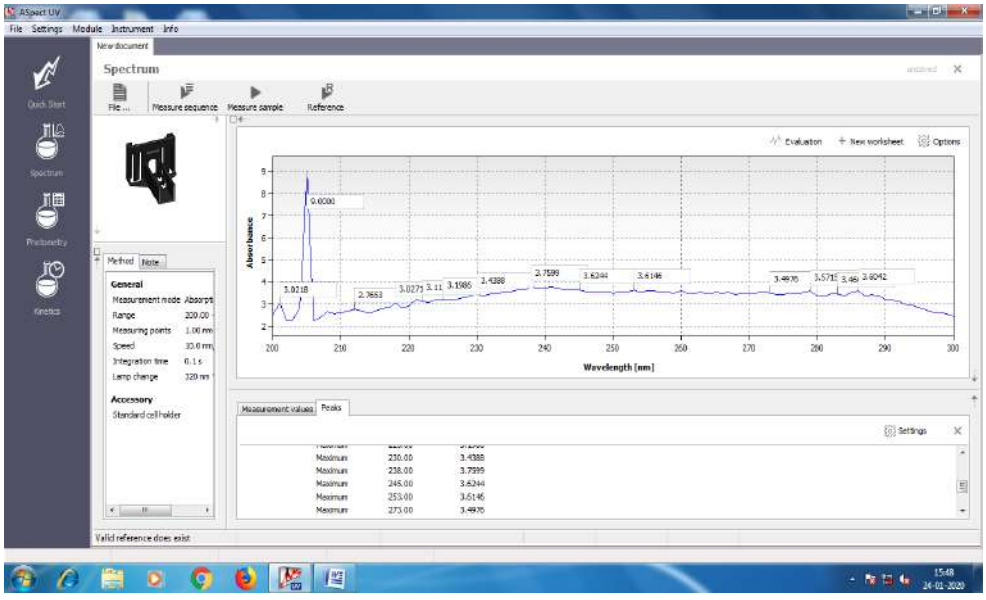


**Figure 22: UV graph of Tiffany Sample 3**

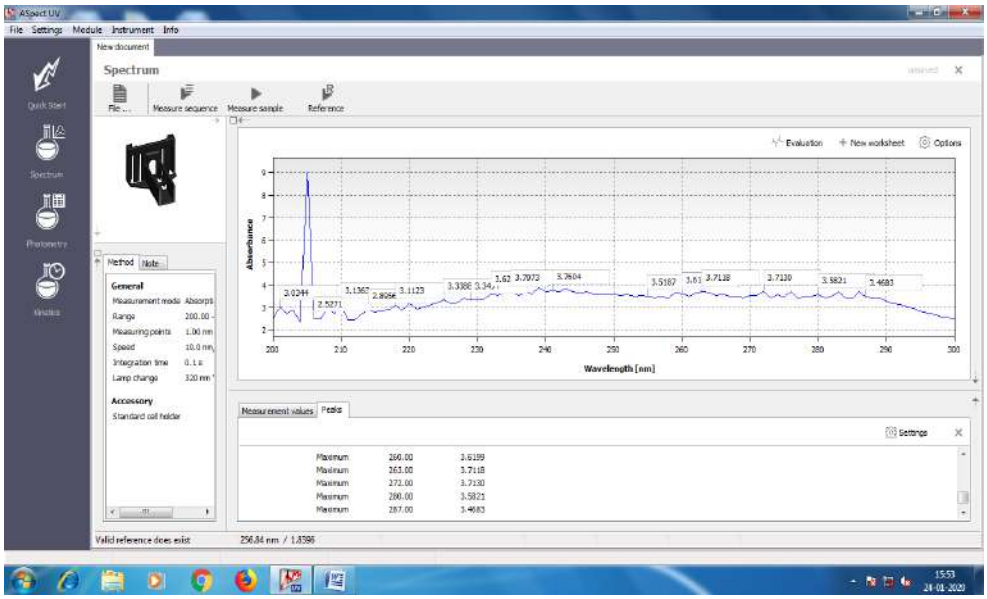


**Figure 23: UV graph of Tiffany Sample 4**

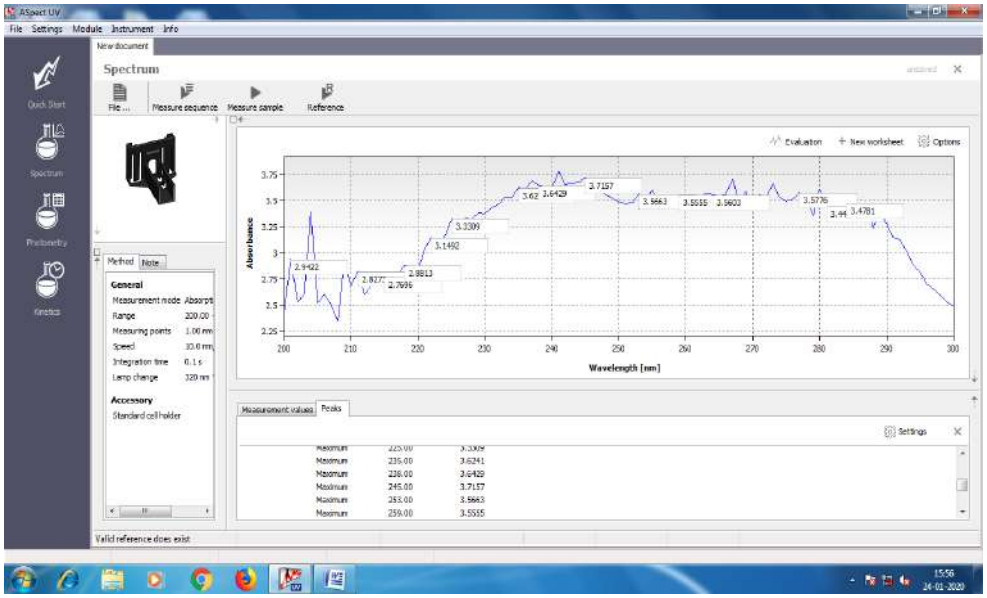


**Figure 24: UV graph of Tiffany Sample 5**

## CHIKO ÉCLAIR

| Samples | Range(nm) | Absorption | Average |
|---|---|---|---|
| Sample 1 | 205 nm | 2.45 | 2.546 |
| Sample 2 | 207 nm | 2.67 | |
| Sample 3 | 208 nm | 2.55 | |
| Sample 4 | 206 nm | 2.39 | |
| Sample 5 | 202 nm | 2.67 | |

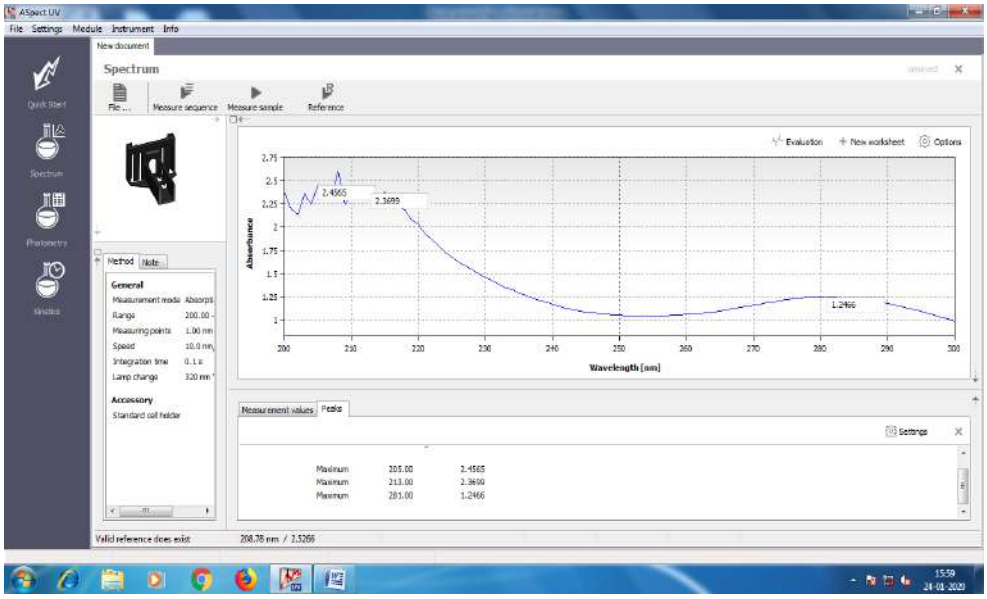


**Figure 25: UV graph of Chiko Éclair Sample1**

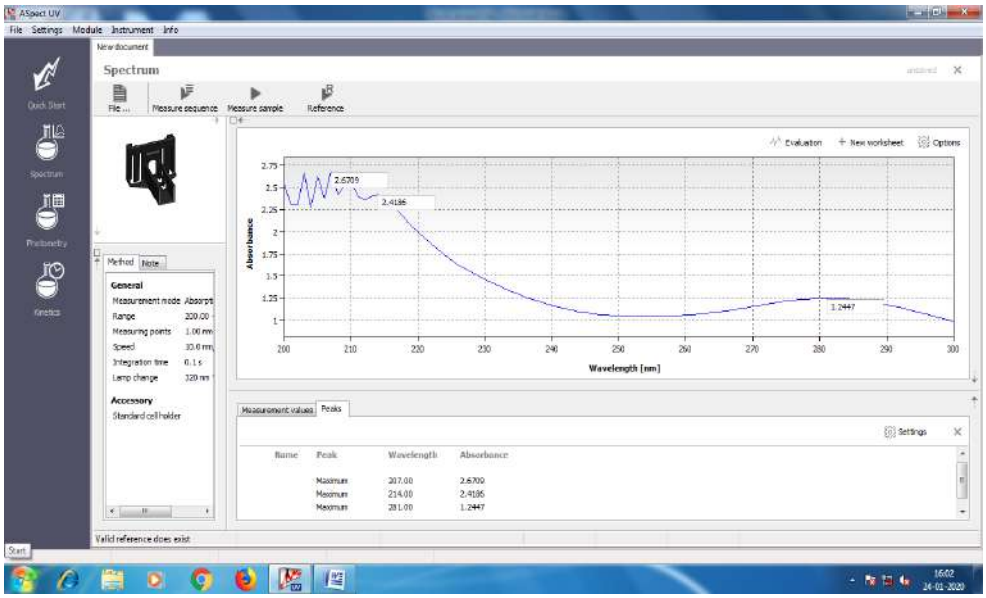


**Figure 26: UV graph of Chiko Éclair Sample2**

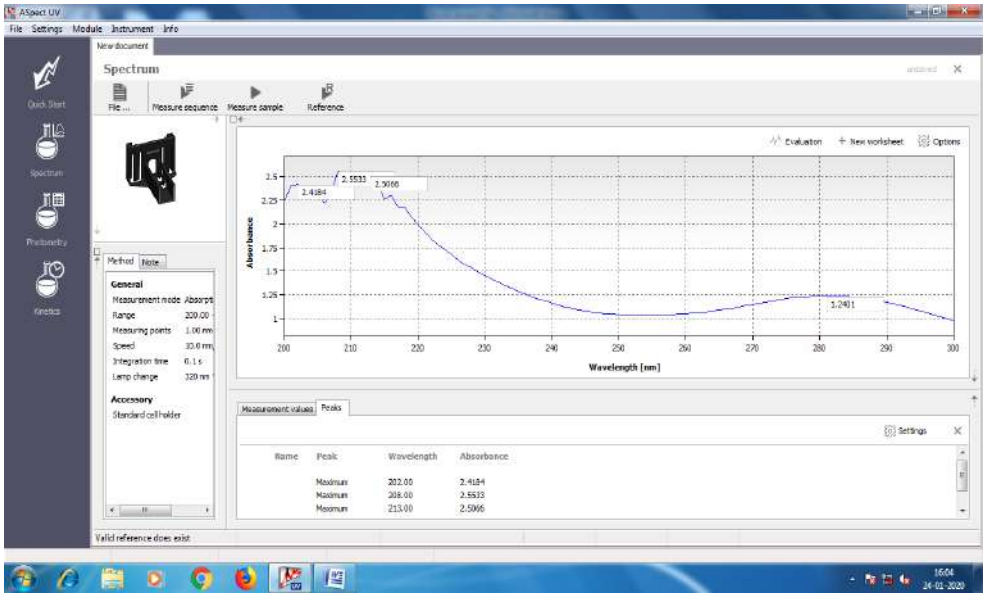


**Figure 27: UV graph of Chiko Éclair Sample3**

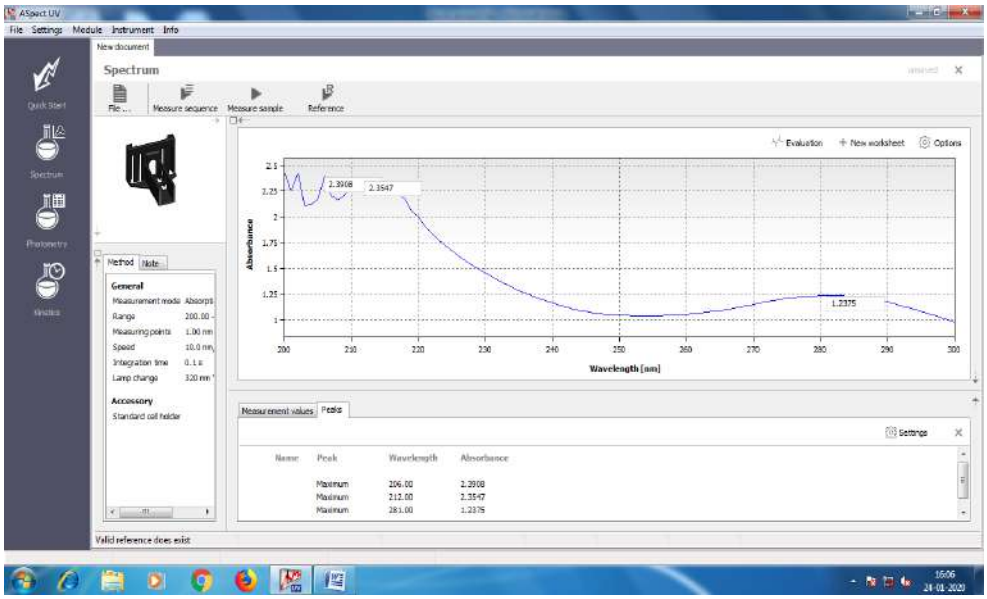


**Figure 28: UV graph of Chiko Éclair Sample4**

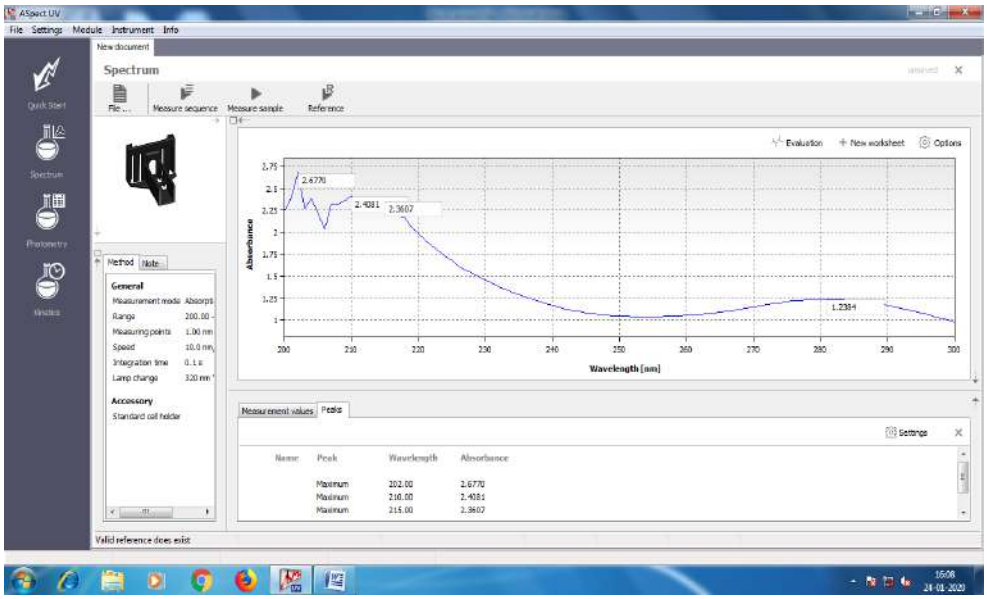


**Figure 29: UV graph of Chiko Éclair Sample 5**

## DARK CHOCOLATE

| Samples | Range(nm) | Absorption | Average |
|---|---|---|---|
| Sample 1 | 219 nm | 2.72 | 2.77 |
| Sample 2 | 21 5 nm | 2.82 | |
| Sample 3 | 222 nm | 2.72 | |
| Sample 4 | 221 nm | 2.79 | |
| Sample 5 | 221 nm | 2.80 | |

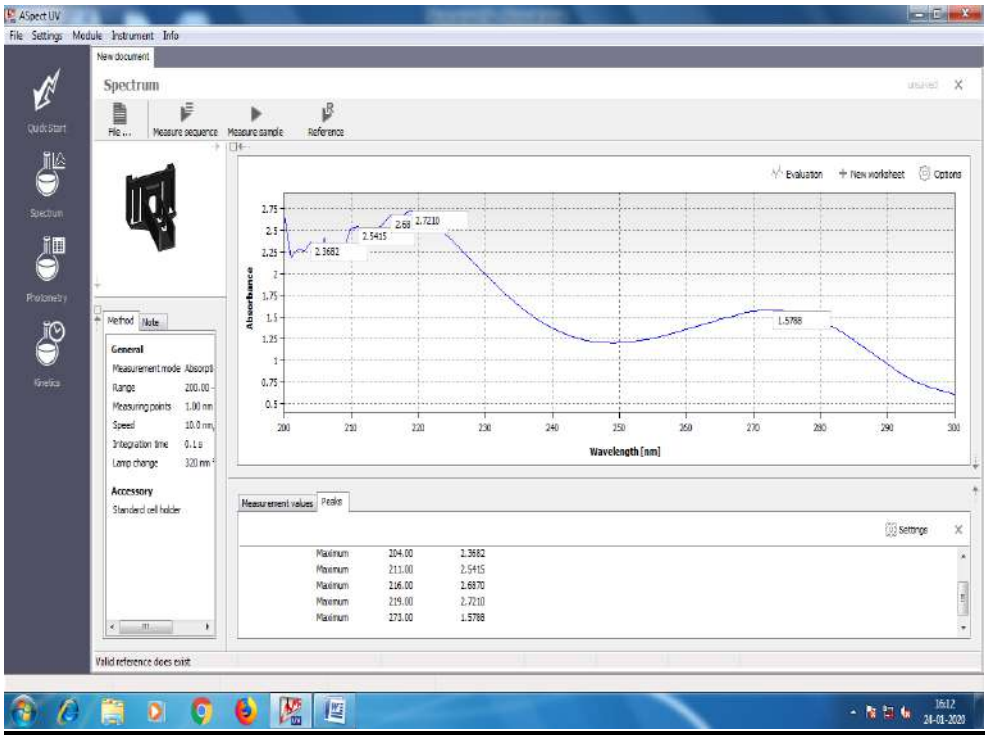


**Figure 30: UV graph of Dark Chocolate sample 1**

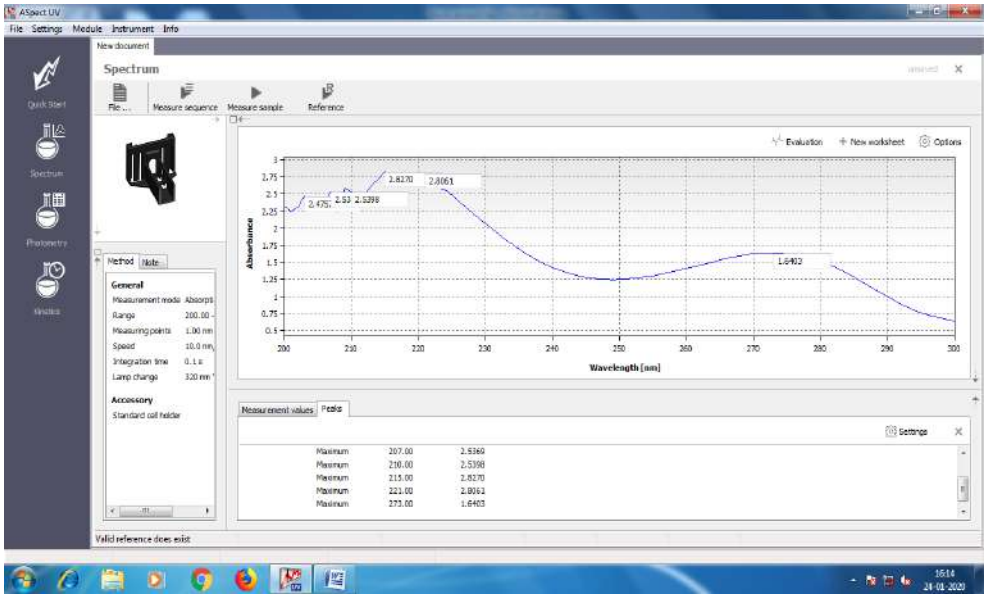


**Figure 31: UV graph of Dark Chocolate sample 2**

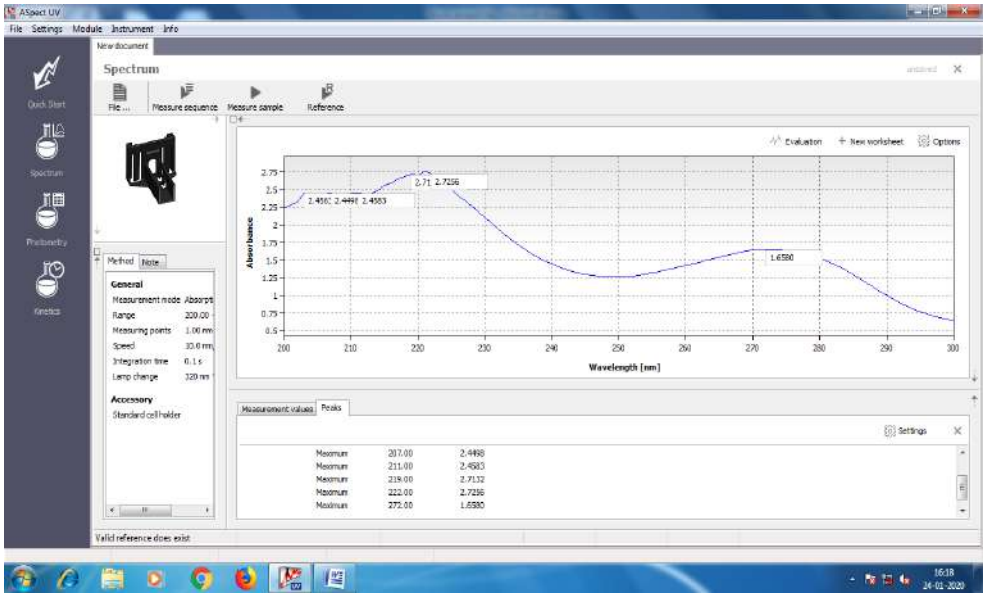


**Figure 32: UV graph of Dark Chocolate sample3**

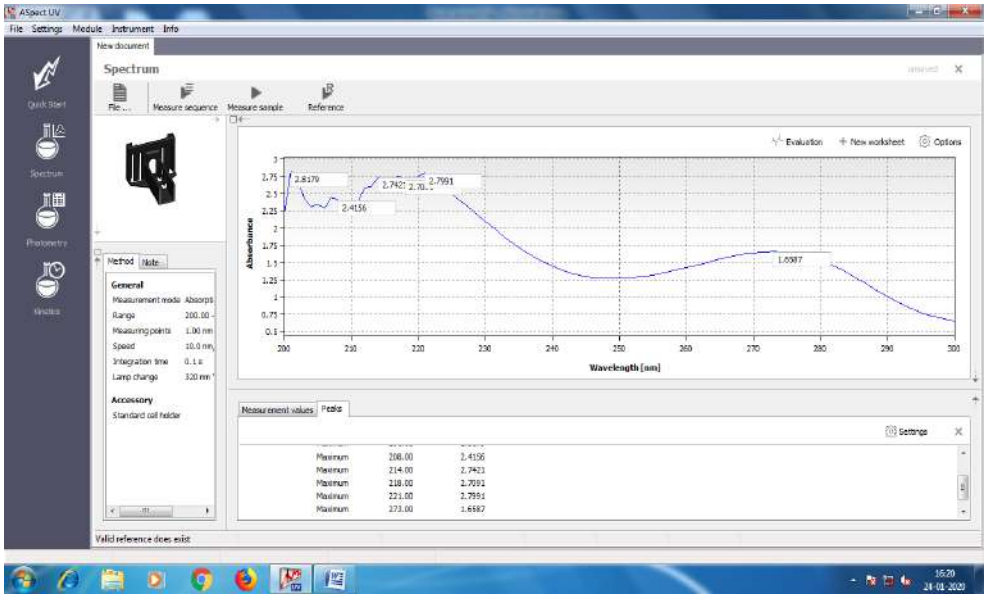


**Figure 33: UV graph of Dark Chocolate sample4**

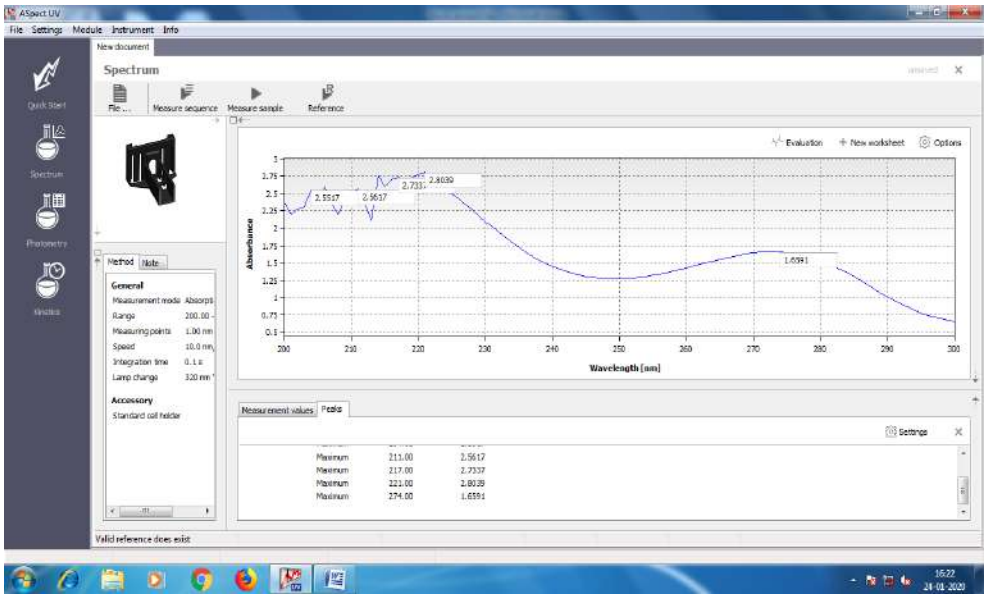


**Figure 34: UV graph of Dark Chocolate sample5**

## GAVOTTES

| Samples | Range(nm) | Absorption | Average |
|---|---|---|---|
| Sample 1 | 230 nm | 3.20 | 3.226 |
| Sample 2 | 228 nm | 3.27 | |
| Sample 3 | 230 nm | 3.22 | |
| Sample 4 | 232 nm | 3.22 | |
| Sample 5 | 232 nm | 3.22 | |

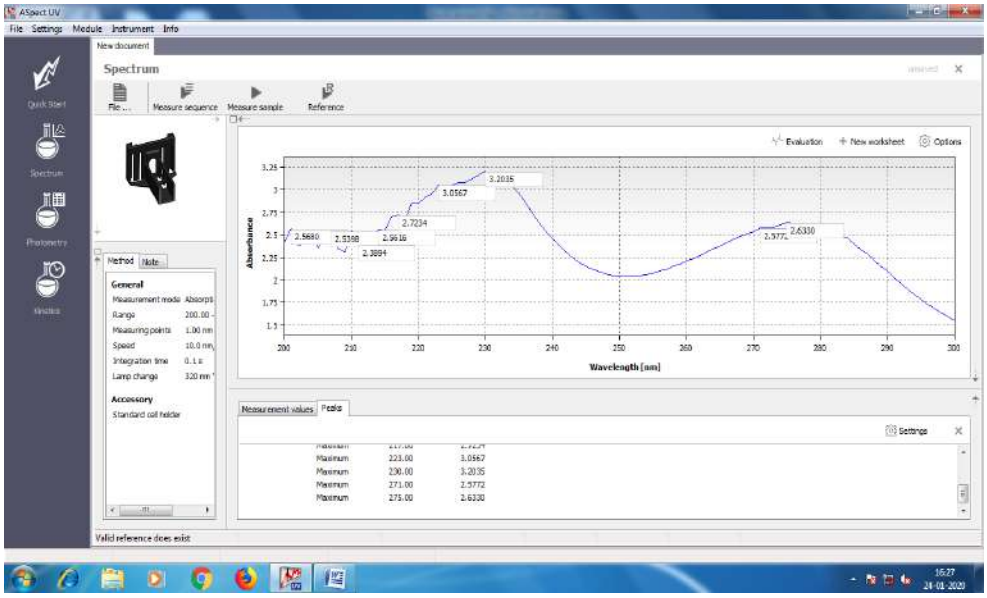


**Figure 35: UV graph of Gavottes sample 1**

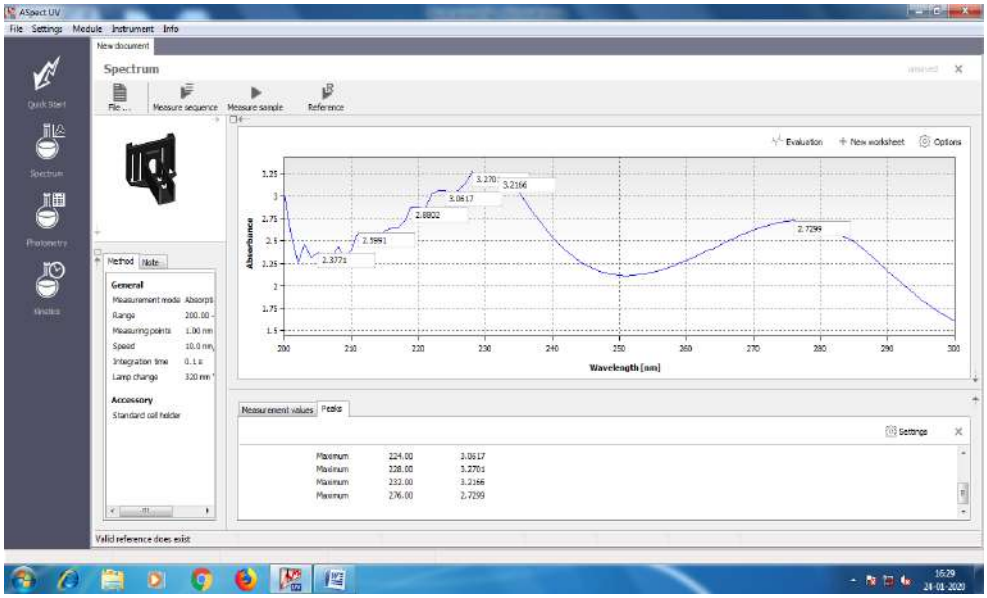


**Figure 36: UV graph of Gavottes sample 2**

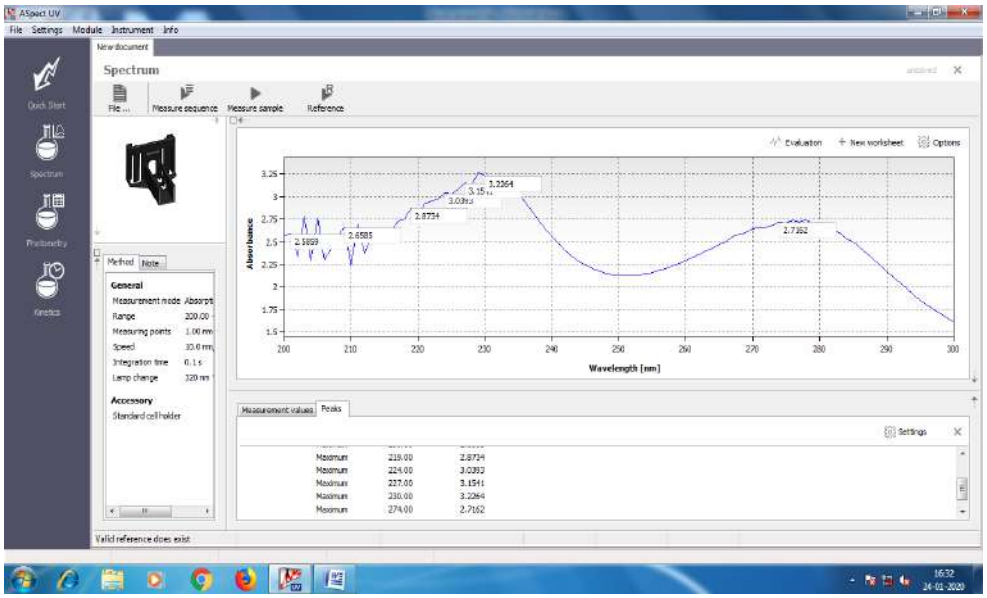


**Figure 37: UV graph of Gavottes sample 3**

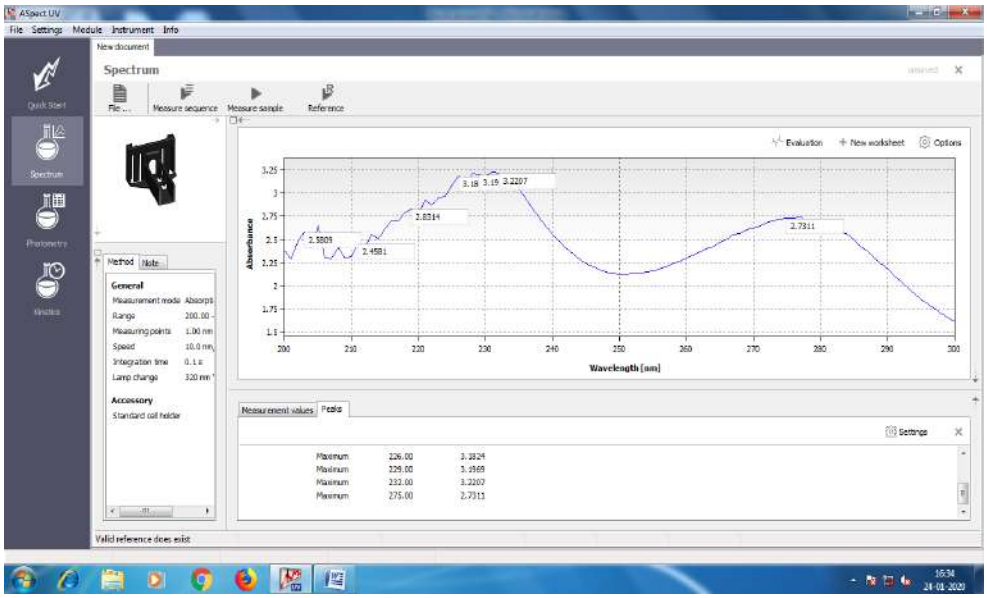


**Figure 38: UV graph of Gavottes sample4**


**Figure 39: UV graph of Gavottes sample 5**

# CHAPTER VI: RESULT AND CONCLUSION

## Result

Average peak of nicotine detected in Galaxy is 232nm at 3.15 absorption Average peak of nicotine detected in Tiffany Éclair is 212nm at 2.54 absorption Average peak of nicotine detected in Chocolala is 237nm at 3.62 absorption Average peak of nicotine detected in Tiffany is 272nm at 3.73 absorption Average peak of nicotine detected in Chiko Éclair is 208nm at 2.55 absorption Average peak of nicotine detected in Dark Chocolate is 221nm at 2.77 absorption Average peak of nicotine detected in Gavottes is 228nm at 3.23 absorption.

## Conclusion

In present study the presence of nicotine is found in Galaxy, Chocolala, Tiffany, Dark Chocolate, and Gavottes but absence of nicotine in Tiffany Éclair and Chiko Éclair.

The study needs to analyse the nicotine in various chocolates by using IR spectroscopy to find out the amount of nicotine present in that particular brand.

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