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Research Article

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UV-VISIBLE SPECTROPHOTOMETER METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF FAMOTIDINE IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Famotidine belongs to H2-receptor antagonist. It is used widely for the treatment of treatment of (GERD) gastro-esophageal reflux disease and gastric ulceration duodenal ulcer, stress ulcer. A simple, efficient spectrophotometric method for the assay of famotidine has been developed for estimation of famotidine formulations. Comparision of three different brands of famotidine (Topcid, Famonext and Famocid) has also been done. The assay is carried out in lambda max at about 320.5nm using water as solvent. Different formulations of drug was dissolved in water to prepare solutions containing famotidine 40 mg. Similarly, a sample of ground tablets of different brand were dissolved in water and dilutions were made in the range of 60-10 µgmL-1. The absorbance of sample preparation was measured at 320.5 nm against the water (blank solvent) and the assay was determined by the absorbance of each brand. Our results reveals that among all the three brands of famotidine (Topcid, famonext and Famocid) famulcer shows highest percentage assay 111.44%. Calibration curves were linear over the range of 12.5-200 µg mL-1with a correlation coefficient ±0.92. Intra and inter-run precision and accuracy results were 98 to 102%. Proposed method was selective, accurate and precise therefore we can use for routine assay as well as quality control and clinical study.

Keywords: Famotidine,Antagonist, Formulations and Spectrophotometry.

INTRODUCTION

UV- Visible Spectroscopy

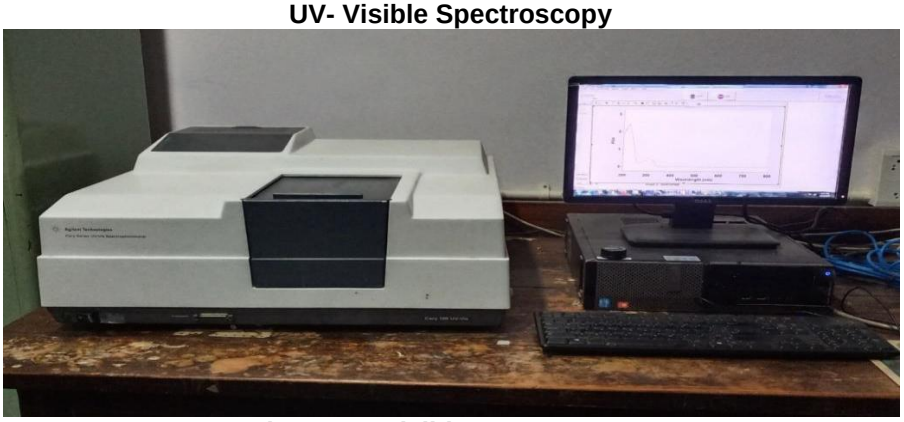

Fig. 1: UV-Visible Spectroscopy

It involves the measurement of amount of ultra-violet radiation absorbed by a substance in the solution. The wavelength between 190-390 nm (practically 200-400 nm) is considered to be UV radiations/ region. Colored compounds absorb in visible range i.e. 400-800 nm.
UV-VISIBLE spectroscopy is a cost-effective, simple, versatile, non-destructive, analytical technique suitable for a large spectrum of organic compounds and some inorganic species. As a function of wavelength, UV-VIS spectrophotometers measure the absorption or transmission of light that passes through a medium.

MATERIALS AND METHODS

Instrumentation

Teccomp UV-2301 double beam UV-Visible spectrophotometer was used to carry out spectral analysis famotidine and the data was recorded by Hitachi software. Standard cuvettes of 10mm path length are used for analysis. Sonicator (1.5L) Ultrasonicator was used to sonicating the standard and formulation sample. Standard and sample drugs were weighed by using Denver electronic analytical balance (SI-234).

Reagents, Standard and samples

Working standard sample famotidine was obtained from well reputed research laboratory, formulation sample was purchased from local pharmacy. Spectrophotometric reagents i.e. methanol and colouring reagents like eriochrome black t, methyl blue, bromocresol green and bromo thymol blue was purchased.

Preparation of standard stock solution

- Take 0.01g of famotidine i.e., 10mg which is to be solubilized in the 10ml of methanol. So, 1000 µg/ml has been prepared as a stock solution.
- Then take 1ml stock solution and then add 9ml oh methanol so 100 µg/ml has been prepared.
- From 100 µg/ml take 1ml and make up with 9ml of methanol so 10 µg/ml has been prepared.
- So, find the λmax of 10 µg/ml i.e., 320.5nm.

Preparation of dilutions

- We need to take 100 µg/ml as stock solution, for 20 µg/ml we require 2ml of stock solution and add 8ml of methanol.
- For 30 µg/ml , we require 3ml of stock solution and to that add 7ml of methanol
- To prepare 40µg/ml, take 4ml of stock solution and add 6 ml of methanol.
- To prepare 50 µg/ml, take 5ml of stock solution and add5ml of methanol.
- To prepare 60µg/ml, take 6ml of stock and add 4ml of the methanol.

Further we have been found the absorbance values of above concentration by taking 3 absorbances of above concentration of the average values.

Dilutions	Absorbances
10 µg/ml	0.083nm
20 µg/ml	0.119nm
30 µg/ml	0.144nm
40 µg/ml	0.175nm
50 µg/ml	0.197nm
60 µg/m	0.232nm

Absorbance values

Preparation of formulation sample

- Famocid
- Famonext
- Topcid

Preparation of the sample

• Take a complete tablet which has equivalent weight of 120mg and make it into fine powder then dissolve in 10ml of methanol by sonication. So,1000µg/ml has been prepared.

- Repeat the same procedure for the remaining brands too.
- From this prepare 100 µg/ml and 10 µg/ml
- Further we need to find absorbance for the concentrations.

UV Spectrophotometric estimation

Selection of solvent for solubility

The drug Famotidine was practically soluble in Water and absorbance of solution was measured. Finally dilutions with water were show improved absorbance compared to other solvents. Hence standard drug was soluble in water and necessary required dilutions were prepared with water as diluents for spectrophotometric estimation.

Selection of wavelength maxima

Suitable maximum absorbance for the estimation of Famotidine was identified by scanning the absorbance in spectrum mode within the wavelength region of 400-200nm in three different dilute solutions. In all the solutions the drug absorb maximum wavelength at 215nm. Hence 215nm was found to be suitable wavelength for the estimation of Famotidine.

Construction of calibration curve

From the prepared standard stock solution, a series of calibration standards were prepared by selected dilutions. From the stock solution, 100µg/ml, 200, 300, 400, 500µg/ml was prepared. The absorbance of the prepared solutions was measured at 215nm against a regent blank. At each concentration a triple readings were measured and mean value was used for the Construction of calibration curve. Calibration curve was constructed by taking concentration of the prepared solution on x-axis and corresponding absorbance on y-axis.

Formulation analysis

The absorbance of the prepared formulation solution in all the brands was measured at 320.5nm in triplets separately. The % assay estimated in the prepared sample solutions by substituting the absorbance values in the equation given below.

$$\%\ \text{Assay} = \frac{\text{At}}{\text{As}} \times \frac{\text{Ws}}{\text{Ds}} \times \frac{\text{Dt}}{\text{Wt}} \times \frac{\text{P}}{100} \times \frac{\text{AW}}{\text{LC}} \times 100$$

Where

At = Absorbance of the sample
As = Absorbance of the Standard
Ws = Weight of the Standard in mg
Wt = Weight of the Sample in mg
Dt = Dilution of the Sample Solution
Ds = Dilution of the Standard Solution
AW = Average Weight of the Tablet
LC = Label Claim of the Tablet
P = % Purity of the Standard Drug

Visible Spectrophotometric estimation

Preparation of Reagents

Eriochrome Black T - Take 0.1 ml of Eriochrome black T dissolve in 100 ml of water.
Methylene Blue - Take 0.1 ml of Methylene blue dissolve in 100 ml of water.
Methylene Orange - Take 0.1 ml of Methylene orange dissolve in 100 ml of water.
Bromocresol Green - Take 0.1 ml of Bromocresol dissolve in 100 ml of water.

Method procedure

Preparation of standard solution

Stock solution of famotidine was prepared by dissolving 100mg of famotidine in 100ml of methanol.

- This solution was sonicated for 5 mins and make up to the volume with methanol, So it was taken as stock solution.
- From the above solution 0.2ml was pippete out and add 0.5 ml of reagent.
- Now dissolve in 9.3 ml of water.
- From the stock solution 0.4ml is taken and we have added reagent about 0.5ml and dissolved in 9.1 ml of water.
- From the stock solution 0.6ml is taken and we have to add the reagent 0.5ml and dissolved in 8.9ml of water.
- From the stock solution 0.8ml is taken and we have to add the reagent 0.5ml and dissolved in 8.7ml of water.
- From the stock solution 1ml is taken and we have to add the reagent 0.5ml and dissolved in 8.5ml of water.
- From the stock solution 1.2 ml is taken and we have to add the reagent 0.5ml and dissolved in 8.3ml of water.

Preparation of blank solution

Take 1 ml from standard stock solution add 0.5 ml of reagent and make up with 8.5 ml of water.

Formulation Assay

From the prepared 10µg/ml of the sample solution, 1ml was taken and the method procedure as describes above was applied. After the development of the color, the absorbance of the separated chloroform layer was measured at 727.0nm against a similar reagent blank. The resultant absorbance values were used for the estimation of Famotidine in the formulation assay. The % assay estimated in the prepared sample solutions by substituting the absorbance values in the equation given below.

$$\%\ \mathbf{Assay} = \frac{\mathbf{At}}{\mathbf{As}} \times \frac{\mathbf{Ws}}{\mathbf{Ds}} \times \frac{\mathbf{Dt}}{\mathbf{Wt}} \times \frac{\mathbf{P}}{\mathbf{100}} \times \frac{\mathbf{AW}}{\mathbf{LC}} \times \mathbf{100}$$

Where

At = Absorbance of the sample
As = Absorbance of the Standard
Ws = Weight of the Standard in mg
Wt = Weight of the Sample in mg
Dt = Dilution of the Sample Solution
Ds = Dilution of the Standard Solution
AW = Average Weight of the Tablet
LC = Label Claim of the Tablet
P = % Purity of the Standard Drug

RESULTS AND DISCUSSION

UV-Method

Wavelength maximum were identified for the Famotidine drug at dilute concentration. Specific wavelength maximum was identified at a wavelength of 320.5nm. Hence 320.5nm was found to be most suitable wavelength for the estimation ofFamotidine. Wavelength scanning result was shown in figure.

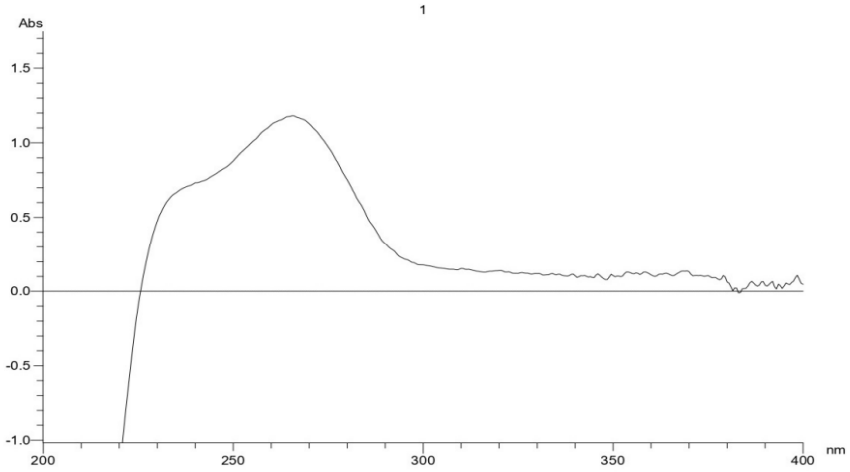

Fig. Wavelength scanning spectrum of Famotidine in UV region

Good linear relation was observed within the prepared concentrations of 10-60µg/ml. regression equation was found to be y = 0.0029x + 0.0573 with correlation coefficient of 0.9963. The high correlation coefficient indicates that the calibration curve was applicable for the estimation ofFamotidine. Results of calibration curve was shown in figure above figure and table.

Table: Calibration Curve Results of Famotidine in UV Method

S. NO	Concentration	Average Absorbance
1	10µg/ml	0.083nm
2	20µg/ml	0.119nm
3	30µg/ml	0.144nm
4	40µg/ml	0.175nm
5	50µg/ml	0.197nm
6	60µg/ml	0.232nm
	Slope Intercept Correlation Coefficient	0.9963

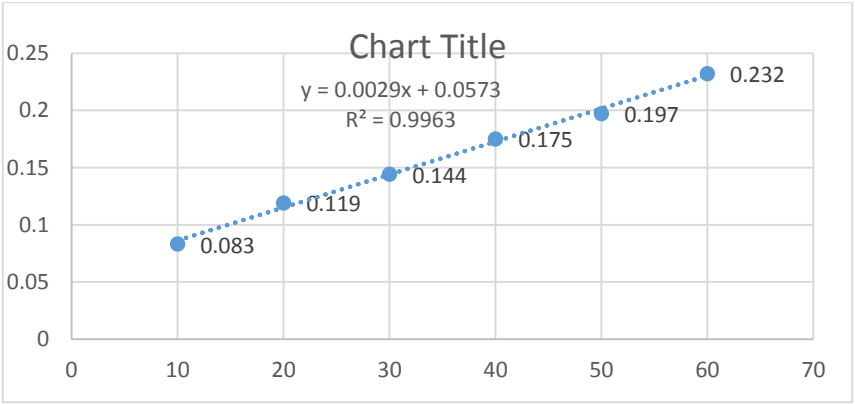

Fig: Calibration Curve of Famotidine in UV Method

Formulation Assay

The absorbance of the prepared formulation solutions was measured and from the resultant sample values % assay was calculated. In the entire brand under study, % assay was found to be 98%. High amount of drug was estimated in Famonext brand. Hence the fallowed method was successfully applied for the estimation of Famotidine. Results of the essay studies were shown in table.

Table: Formulation results of Famotidine in UV Method

S.NO	Brand	Dosage	Amount Prepared	Absorbance Found	%Assay
1	Topcid	40mg	10µg/ml	0.166	93.7%
2	Famonext	40mg	20µg/ml	0.169	98%
3	Famocid	40mg	30µg/ml	0.171	96.2%

Visible Spectrophotometric estimation

RESULTS AND DISCUSSION

From the prepared color developed solution of the Famotidine one solution was taken and the absorbance of the solution was scanned in the visible regioni.e800nm-400nm against a similarly prepared reagent blank. At a wavelength of 727.0nm was found to be most suitable for the estimation of Famotidine Wavelength scanning spectrum was shown in figure 6.3.

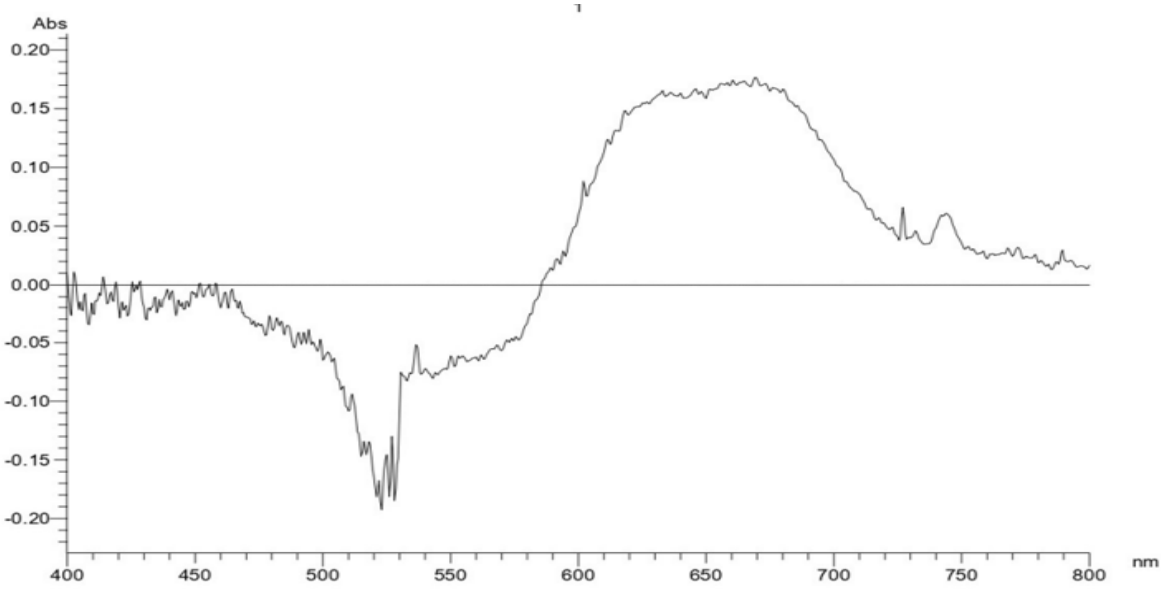

Fig: Wavelength scanning spectrum of Famotidine in Visible region

Six points calibration curve was constructed with in the concentration range of 0.2-1.2µg/ml. regression equation was found to be y = 0.004x - 0.009 with a correlation of 0.996. Results of the calibration curve was shown in table 4 and calibration curve was shown in below figure.

Table: Calibration Curve Results of Famotidine in Visible Method

S. NO	Concentration	Average Absorbance
1	0.2µg/ml	0.085nm
2	0.4µg/ml	0.108nm
3	0.6µg/ml	0.136nm
4	0.8µg/ml	0.168nm
5	1µg/ml	0.197nm
6	1.2µg/ml	0.225nm
	Slope Intercept Correlation Coefficient	0.9982

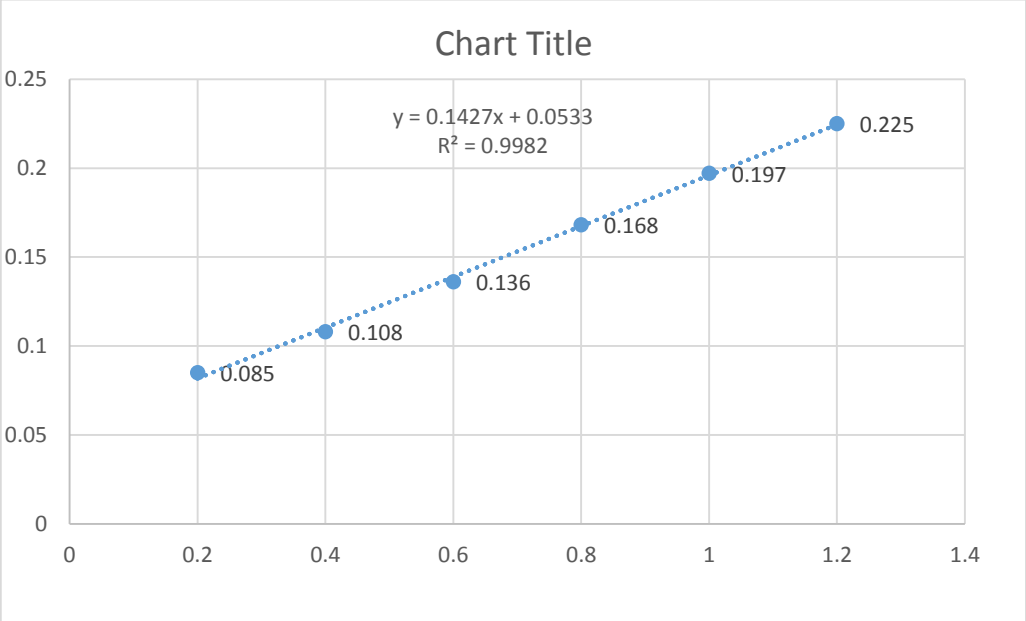

Fig: Calibration Curve of Famotidine in Visible Method

Formulation Assay

The absorbance of the prepared formulation solutions was measured and from the resultant sample values % assay was calculated. In the entire brand under study, % assay was found to be 80%. High amount of drug was estimated inTopcidbrand. Hence the fallowed method was successfully applied for the estimation of Famotidine. Results of the essay studies were shown in table.

Table: Formulation results of Famotidine in Visible Method

S.NO	Brand	Dosage	Amount Prepared	Absorbance found	%Assay
1	Topcid	40mg	10µg/ml	0.514	80.52%
2	Famonext	40mg	10µg/ml	0.499	77.9%
3	Famocid	40mg	10µg/ml	0.503	78.6%

CONCLUSION

Famotidine is used to treat ulcers of the stomach and intestines and to prevent intestinal ulcers from coming back after they have healed. It works by decreasing the amount of acid your stomach makes. It relieves symptoms such as cough that doesn't go away, stomach pain, heartburn, and difficulty swallowing. Famotidine belongs to a class of drugs known as H2 blockers. This medication is also available without a prescription.

In this thesis, we estimate the drug Famotidine in market tablet brands Topcid, Famonext, Famocid of 40mg/ml by UV and Visible spectrophotometry.

In UV region drug was estimated at 320.5nm using methanol as diluents and in visible region the color was developed using Eriochrome Black T reagent. The maximum absorbance of the developed violet color was found to be 727.0nm. Beers law equation was found to be y = 0.0029x + 0.0573 for UV and y = 0.0143x + 0.0533 for Visible method. In both these methods the drugs were estimated more than 93% assay.

BIBLIOGRAPHY

1. Liu P-F, Avramova LV and Park C. Revisiting absorbance at 230nm as a protein unfolding probe. Anal Biochem. 2009;389(2):165-170. doi:10.1016/j.ab.2009.03.028.
2. Kalb V and Bernlohr R. A New Spectrophotometric Assay for Protein in Cell Extracts. Anal Biochem. 1977;82:362-371. doi:10.1016/0003-2697(77)90173-7.
3. Bosch Ojeda C and Sanchez Rojas F. Recent applications in derivative ultraviolet/visible absorption-spectrophotometry: 2009–2011. Microchem J. 2013;106:1-16. doi:10.1016 /j.microc.2012.05.012.
4. Domingo C and Saurina J. An overview of the analytical characterization of nanostructured drug delivery systems: Towards green and sustainable pharmaceuticals: A review. Anal ChimActa. 2012;744:8-22. doi:10.1016/j.aca.2012.07.010.
5. Gaikwad J, Sharma S and Hatware KV. Review on Characteristics and Analytical Methods of Tazarotene: An Update. Crit Rev Anal Chem.2020;50(1):90-96. doi:10.1080 /10408347.2019.1586519.
6. Gendrin C, Roggo Y and Collet C. Pharmaceutical applications of vibrational chemical imaging and chemometrics: A review. J Pharm Biomed Anal. 2008;48(3):533-553. doi:10.1016/j.jpba.2008.08.014
7. Lourenço ND, Lopes JA and Almeida CF, Sarraguça MC, Pinheiro HM. Bioreactor monitoring with spectroscopy and chemometrics: a review. Anal Bioanal Chem. 2012;404(4):1211-1237. doi:10.1007/s00216-012-6073-9.
8. Sánchez Rojas F and Bosch Ojeda C. Recent development in derivative ultraviolet/visible absorption spectrophotometry: 2004–2008. Anal Chim Acta. 2009;635(1):22-44. doi:10.1016/j.aca.2008.12.039.
9. Stevenson K, McVey AF, Clark IBN, Swain PS and Pilizota T. General calibration of microbial growth in microplate readers. Sci Rep. 2016;6(1):38828. doi:10.1038/srep38828.
10. Tadesse Wondimkun Z. The Determination of Caffeine Level of Wolaita Zone, Ethiopia Coffee Using UV-visible Spectrophotometer. Am J Appl Chem. 2016;4(2):59. doi:10.11648/j.ajac.20160402.14.
11. Yu J, Wang H, Zhan J and Huang W. Review of recent UV–Vis and infrared spectroscopy researches on wine detection and discrimination. Appl Spectrosc Rev. 2018;53(1):65-86. doi:10.1080/05704928.2017.1352511.
12. Leong Y, Ker P and Jamaludin M. UV-Vis Spectroscopy: A New Approach for Assessing the Color Index of Transformer Insulating Oil. Sensors. 2018;18(7):2175. doi:10.3390/s18072175.
13. Brown JQ, Vishwanath K, Palmer GM and Ramanujam N. Advances in quantitative UV–visible spectroscopy for clinical and pre-clinical application in cancer. Curr Opin Biotechnol. 2009;20(1):119-131. doi:10.1016/j.copbio.2009.02.004.
14. Pinheiro HM, Touraud E and Thomas O. Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry wastewaters. Dyes Pigm. 2004;61(2):121-139. doi:10.1016/j.dyepig.2003.10.009.
15. Kristo E, Hazizaj A and Corredig M. Structural Changes Imposed on Whey Proteins by UV Irradiation in a Continuous UV Light Reactor. J Agric Food Chem. 2012;60(24):6204-6209. doi:10.1021/jf300278k.
16. Lange R and Balny C. UV-visible derivative spectroscopy under high pressure. Biochim Biophys Acta BBA - Protein Struct Mol Enzymol. 2002;1595(1-2):80-93. doi:10.1016/S0167-4838(01)00336-3.