METHOD DEVELOPMENT AND VALIDATION OF RIBOFLAVIN OBTAINED FROM THE EXTRACTION OF KIWI FRUIT BY UV SPECTROPHOTOMETRY

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ABSTRACT:

The present study was aimed to develop and validate a simple, accurate, precise, reproducible UV-Visible spectrophotometric method for the estimation of Riboflavin present in kiwi fruit. The solvent used in the experiment was methanol and hot water in the ratio 35:65v/v%. Absorption maximum (λmax) of the drug was found to be 370 nm. The Beer's law was obeyed in the range of 50-500 µg/mL. The method was shown linear in the mentioned concentrations having line equation y = 0.001x+ 0.029 with correlation coefficient r^2 of 0.994. The amount of Riboflavin present in kiwi fruit was found to be 0.04mg/ml. The recovery values for Riboflavin present in kiwi fruit ranged from 98.57%-99.16%.The percent relative standard deviation (RSD %) of interday precision was 0.949% and intraday precision was 0.573%. The limit of detection and limit of quantification was 0.079µg/mL and 0.154µg/mL. The percent relative standard deviation of robustness and ruggedness of the method was 0.055-0.239%. Hence, proposed method was precise, accurate and cost effective. This method could be applicable for quantitative determination of the Riboflavin present in kiwi fruit.

Introduction

Riboflavin also known as vitamin B_2. It is a vitamin found in food and used as a dietary supplement[1,3]. Food sources includes eggs, green vegetables, milk and other dairy product, meat, mushrooms, and almonds[3]. Some countries require its addition to grains[3,4]. As a supplement it is used to prevent and treat riboflavin deficiency and prevent migraines[1,3]. It is required by the body for cellular respiration[1].

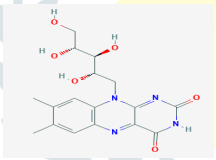

Fig 1:Structure of riboflavin

Kiwifruit:

Kiwifruit is botanically known as Actinidia deliciosa[5]. The genus Actinidia belongs to the family Actinidiaceae, to the order Ericales, to the class Magnoliopsida and to the division Magnoliophyta. There are dozens of species comprising the genus Actinidia[6]. The most common kiwifruit species grown commercially is Actinidia deliciosa (green kiwi), even though many varieties of this fruit are produced by other cultivars or by another kind of plants, such as Actinidia chinensis (yellow kiwi) and the Actinidia Kolomikta or the Actinidia arguta (baby kiwi)[7]. In fact, the world production of A. arguta already corresponds to 17% of the total kiwi produced worldwide, with A. deliciosa and A. chinensis being the most produced species, with 37% and 31%, respectively, and A. kolomitka and other inter specific hybrids the species with the lowest world production, with 8% and 7%, respectively[8].

The Actinidia is native to North East Asia, particularly South China. The most common cultivars of kiwifruit are oval, about the size of a large hen's egg (5–8 cm / 2–3 in long and 4.5–5.5 cm / 1¾–2 in diameter). It has a fibrous, dull brown-green skin and bright green or golden flesh with rows of tiny, black, edible seeds. The fruit has a soft texture and a unique flavour, is a commercial crop in several countries, mainly in Italy, China, and New Zealand. Also known as the Chinese gooseberry, the fruit was renamed for export marketing reasons in the 1950s; briefly to melonette, and then by New Zealand exporters to kiwi fruit. This latter name comes from thekiwi- a brown flightless bird and New Zealand's national symbol, and also a colloquial name for

the New Zealand people [9]. It has been reported that folk remedy for adult diseases, such as potent anti-hepatotoxic, anti-pyorrheal and gingival inflammation, was observed in the roots of Actinidia deliciosa[10]. The genus Actinidia (Actinidiaceae) are widely used in Chinese folk medicines to treat such diseases as hepatitis, edema, rheumatoid arthritis, gastric cancer and breast cancer etc[11]. Actinidia deliciosa is distributed in west China, and showed to have anti-tumor and protective effects on acute hepatic injury in biological arrays [12-16]. Kiwi fruit is often reported to have mild laxative effects, due to its significant levels of dietary fiber [17]. Kiwi fruit components, possibly involving vitamin E and omega-3 fatty acids from its numerous edible seeds, have potential properties of a natural blood thinner [18]. Kiwi fruit is a natural source of carotenoids, such as provitamin A beta-carotene,[19] lutein and zeaxanthin [20]. The fruits, stems and roots are diuretic, febrifuge and sedative [21]. They are used in the treatment of stones in the urinary tract, rheumatoid arthralgia, cancers of the liver and oesophagus [21]. Usually Actinidia deliciosa is eaten fresh; however it can also used in beverages, desserts, and as a flavoring. The fruits are very high in vitamin C, along with containing vitamins A and E, also it contains considerable potassium [21]. Kiwi fruit is a rich source of vitamin C. It also contains vitamin E, and a small amount of vitamin A. Kiwi fruit is a good source of flavonoid antioxidants. The kiwifruit seed oil contains on average 62% alpha-linolenic acid, an omega-3 fatty acid [22]. Sliced kiwifruit has long been regularly used as a garnish atop whipped cream on New Zealand's national dessert, the Pavlova. It can also be used in curry [22]. Apart from this, Actinidia deliciosa is also good source of Dietary fiber, Protein, Calcium, Iron, various Vitamins like Thiamine (Vitamin B1), Riboflavin (Vitamin B2), Niacin (Vitamin B3), Vitamin B6, Folate (Vitamin B9), Vitamin E, Vitamin K.

Fig 2:Kiwifruit

Preparation of reagents:
Preparation of diluents: To 100 ml volumetric flask add 35ml of hot water and 65ml of methanol to maintain 35:65
Preparation of standard solution
Preparation of solution A: 1 gm of Standard Riboflavin is weighed and it was added to 100ml of diluent
Preparation of solution B: Pipette out 10ml of Solution A and dilute to 100ml with diluent to prepare 1000ug/ml concentration. By appropriate of solution B with the diluents, different solutions with different concentrations (50, 100, 200, 300, 400 and 500ug/ml) of riboflavin were prepared futher for the development method.

Method Development
Determination of wavelength of maximum absorption:
Preparation of solution A: 5 gms of kiwi fruit is weighed accurately and it was added to 100 ml of diluent.
Preparation of solution B: Pipette out 10 ml of solution A and make upto 100ml with diluent in volumetric flask to prepare 1000 ug concentration. By appropriate of solution B with the diluents, different solutions with different concentrations (50, 100, 200, 300, 400 and 500ug/ml) of test solution was taken and scanned in the range of 200- 400 nm to determine the wave length for maximum absorbance. And it was found that the maximum absorbance at 370nm.

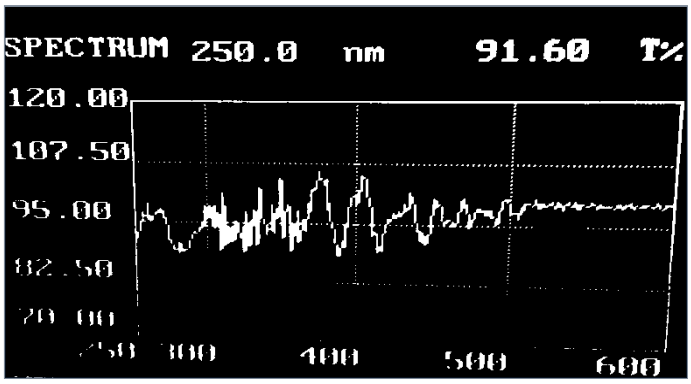

Fig 3: Scanning of Wavelength

Method validation: The proposed method was validated for different parameters like linearity, precision, accuracy, specificity, robustness, LOD, LOQ and assay.

Linearity Study: The linearity was determined by plotting concentration against corresponding absorbance. Standard stock solutions, 1000µg/mL were further diluted with the diluent to obtain 50µg/mL-500 µg/mL solutions. The calibration curves were constructed by plotting absorbance versus concentration and the regression equations were calculated.

Table 1: Calibration curve data of riboflavin

S. No	Concentration(µg/ml)	Absorbance
1	0	0
2	50	0.102
3	100	0.214
4	200	0.389
5	300	0.538
6	400	0.699
7	500	0.832

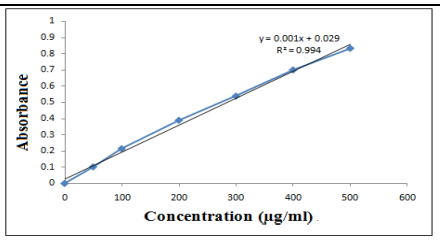

Fig 4: Calibration curve of Riboflavin

Precision

Intra-day precision study: Solution B was diluted further to obtain 50- 500ug/ml concentration. Six replicates were measured and the percentage RSD was calculated.

Inter-day precision study: The selected concentrations for the intra-day precision study were again analysed for consecutive three days and the percentage RSD was calculated.

Table 2: Intraday and Inter day data of riboflavin

Sample No	Intraday precision	Inter day precision
1	102.8	98.6
2	101.7	99.5
3	101.6	98.3
4	102.4	97.6
5	101.5	99.6
6	101.3	100.1
Mean	**101.88**	**98.95**
SD	**0.584**	**0.939**
%RSD	**0.573**	**0.949**

Accuracy and Recovery Studies: Accuracy of the method was calculated by recovery studies at three different levels (50%, 100% and 150%) by standard addition method to study the accuracy of the method and to check the interference from excipients.The first recovery study was conducted on the excipients mixture (placebo) prepared by adding accurately weighed amounts of kiwi fruit to the excipient mixture and calculating the percentage recovery in each case.

Table 3: Results of Recovery study

%Recovery level	% Recovery	Mean % Recovery	SD	% RSD
50%	98.64	98.63	0.015	0.015
	98.63			
	98.61			
100%	98.53	98.57	0.032	0.033
	98.58			
	98.59			
150%	99.12	99.16	0.032	0.032
	99.18			
	99.17			

Specificity in the presence of excipients: The specificity test was carried out using only excipients. Spectra for blank and sample were measured for different time intervals and compared.

Table 4: Results of specificity

Time	Standard	Sample
0	0.923	0.922
2	0.921	0.920
4	0.922	0.921
6	0.924	0.922
Mean	**0.9225**	**0.921**
SD	**0.012**	**0.095**
%RSD	**0.139**	**0.104**

Robustness: The robustness of an analytical products interfered with the quantification of the drug. Procedure is the measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It was determined by carrying out the analysis by at different wavelengths i.e. at 371nm, 372nm and 373nm. The absorbance was measured and assay was calculated for six times.

Table 5: Results of Robustness

S.No	Wave lengths		
	371nm	**372nm**	**373nm**
1	0.732	0.736	0.738
2	0.732	0.736	0.737
3	0.73	0.736	0.736
4	0.733	0.737	0.737
5	0.734	0.736	0.737
6	0.735	0.736	0.737
Mean	**0.732**	**0.736**	**0.737**
SD	**0.001**	**0.004**	**0.007**
%RSD	**0.239**	**0.055**	**0.096**

Limit of Detection (LOD) and Limit of Quantitation (LOQ): LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the ten replicate determinations of same concentration , standard deviation (SD) of the responses was calculated. Limit of detection can be calculated by using the following formula:

LOD = 3.3 σ /S=**2.08 µg /ml**

Limit of quantitation can be calculated based on standard deviation of the response and the slope.

LOQ = 10 σ/S=**6.32 µg /ml**

Where σ = Standard deviation of the response; S = Slope of the calibration curve.

Assay of kiwi fruit formulations: To analyze the concentration of kiwi fruit in the vial, a portion of powder equivalent to 100mg of kiwi fruit was transferred in 100ml volumetric flask and was diluted with Solution B. This solution was further diluted with water to get final concentration of 10µg/mL of kiwi fruit. The % assay of the drug was calculated. All determinations were conducted by thrice time.

Table 6: Results of Assay

Drug	Declared Concentration	Amount found Concentration	Amount found (%)	%RSD
Sample 1	10ug/ml	4.23±0.01	42.3±0.01	0.23
Sample 2	10ug/ml	4.22±0.01	42.2±0.01	0.13
Sample 3	10ug/ml	4.24±0.01	42.3±0.01	0.49

RESULTS AND DISCUSSION

The method discussed in the present work provides a convenient and accurate way for analysis of kiwi fruit. The different concentrations were scanned and the wavelength of maximum absorption was found at 370nm. The drug obeyed the Beer's law with the concentration range 50-500ug/ml having line equation y = 0.001x + 0.029 with correlation coefficient r^2 of 0.994 and represented excellent linear relationship of the newly developed method. The amount of Riboflavin present in kiwi fruit was found to be 0.04mg/ml. The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solution (10 µg/mL) for 6 times and the values of LOD and LOQ were found to be 2.08µg/mL and 6.32µg/mL respectively. The recovery values for Riboflavin present in kiwi fruit ranged from 98.57% - 99.16%.The percent relative standard deviation (RSD %) of interday precision was 0.949% and intraday precision was 0.573%. The limit of detection and limit of quantification was 0.079µg/mL and 0.154µg/mL. The percent relative standard deviation of robustness and ruggedness of the method was 0.055 – 0.239%. Hence, proposed method was precise, accurate and cost effective. This method could be applicable for quantitative determination of the Riboflavin present in kiwi fruit.

CONCLUSION

The method discussed in the present work provides a convenient and accurate way for analysis of kiwi fruit. The different concentrations were scanned and the wavelength of maximum absorption was found at 370nm. The drug obeyed the Beer's law with the concentration range 50-500ug/ml having line equation y = 0.001x + 0.029 with correlation coefficient r^2 of 0.994 and represented excellent linear relationship of the newly developed method. The amount of Riboflavin present in kiwi fruit was found to be 0.04mg/ml. The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solution (10 µg/mL) for 6 times and the values of LOD and LOQ were found to be 2.08µg/mL and 6.32µg/mL respectively. The recovery values for Riboflavin present in kiwi fruit ranged from 98.57% - 99.16%.The percent relative standard deviation (RSD %) of interday precision was 0.949% and intraday precision was 0.573%. The limit of detection and limit of quantification was 0.079µg/mL and 0.154µg/mL. The percent relative standard deviation of robustness and ruggedness of the method was 0.055-0.239%. Finally we conclude that the proposed method was precise, accurate and cost effective. This method could be applicable for quantitative determination of the Riboflavin present in kiwi fruit.

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