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Method development and validation of quercetin obtained from the extraction of almond leaves by UV spectrophotometry and FTIR

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Abstract
The present study was aimed to develop and validate a simple, accurate, precise, reproducible UV-Visible spectrophotometric method and FTIR for the estimation of quercetin present in extract of almond leaves. The solvent used in the experiment was distilled water. Absorption maximum (λmax) of the drug was found to be 375 nm. The Beer's law was obeyed in the range of 25-125 µg/mL. The method was shown linear in the mentioned concentrations having line equation y = 0.008x+ 0.0115 with correlation coefficient r^2 of 0.9972. The amount of quercetin present in almond leaves extract was found to be 105 ug/ml. The recovery values for quercetin present in almond extract powder ranged from 98.57%-99.16%.The percent relative standard deviation (RSD %) of interday precision was 0.938% and intraday precision was 0.628%. The limit of detection and limit of quantification was 0.413µg/mL and 1.25µg/mL. The percent relative standard deviation of robustness and ruggedness of the method was 0.136 – 0.543. Hence, proposed method was precise, accurate and cost effective. This method could be applicable for quantitative determination of the quercetin present in almond leaves extract.

Keywords: almond leaves, quercetin, spectrophotometry, I

Introduction

Fig 1

The Indian almond (Terminalia Catappa L.) tree is abundant in tropical areas and is also found in some parts of the USA. Its leaves, fruit and bark have been used for medicinal purposes, including antioxidant and antimicrobial, owing to the high polyphenolic compounds content including tannins [11, 12]. The main tannin component in Indian Almond is reported to be punicalagin, 13 classified as a hydrolyzable tannin that is the leaves contain phytosterols, saponins, flavonoids such as quercetin and kaempferol as well as tannins such as tercatin, punicalin and punicalagin. The leaves can be used for treating and preventing diarrhoea, dysentery, Cancer and liver diseases. Indian almond leaves come from the *Terminalia catappa* tree. The leaf of this tree is especially known for its ability to act as a natural medicine and aquarium water conditioner for betta fish and shrimp tanks when the leaf has emerged in water for extended periods of time. Indian almond leaves are said to help combat fungus and bacterial problems like fin rot and can further help prevent fish from getting stressed by mimicking the water they are naturally found in. The *Terminalia catappa* tree grows throughout the tropical regions of Asia, Australia, and Africa. As we'll discuss later, the leaves fall from the tree and into the water. Tannins then leach out of the leaves into the water, adding hues of yellow and brown while lowering the pH. Indian almond leaves are usually harvested by simply picking them up off the ground leaf by leaf. After drying them properly, the leaves can then be added into the water or the tank. Almonds are sensitive souls, and are fussy about their growing conditions, which unfortunately means they can be about as challenging to grow as they are delicious.

The almond (*Prunus dulcis*, syn. *Prunus amygdalus*) is a species of tree native to Iran and surrounding countries [3, 4] but widely cultivated elsewhere. The almond is also the name of the edible and widely cultivated seed of this tree. Within the genus *Prunus*, it is classified with the peach in the subgenus *Amygdalus*, distinguished from the other subgenera by corrugations on the shell (endocarp) surrounding the seed. [*Citation needed*]

The fruit of the almond is a drupe, consisting of an outer hull and a hard shell with the seed, which is not a true nut, inside. Shelling almonds refers to removing the shell to reveal the seed. Almonds are sold shelled or unshelled. Blanched almonds are shelled almonds that have been treated with hot water to soften the seedcoat, which is then removed to reveal the white embryo.

The almond is native to Iran and surrounding countries [3, 4]. It was spread by humans in ancient times along the shores of the Mediterranean into northern Africa and southern Europe, and more recently transported to other parts of the

world, notably California, United States [4]. The wild form of domesticated almond grows in parts of the Levant [12].

Quercetin

Is a polyphenolic flavonoid with potential chemopreventive activity. Quercetin, ubiquitous in plant food sources and a major bioflavonoid in the human diet, may produce antiproliferative effects resulting from the modulation of either EGFR or estrogen-receptor mediated signal transduction pathways. Although the mechanism of action of action is not fully known, the following effects have been described with this agent *in vitro*: decreased expression of mutant p53 protein and p21-ras oncogene, induction of cell cycle arrest at the G1 phase and inhibition of heat shock protein synthesis. This compound also demonstrates synergy and reversal of the multidrug resistance phenotype, when combined with chemotherapeutic drugs, *in vitro*. Quercetin also produces anti-inflammatory and anti-allergy effects mediated through the inhibition of the lipoxygenase and cyclooxygenase pathways, thereby preventing the production of pro-inflammatory mediators.

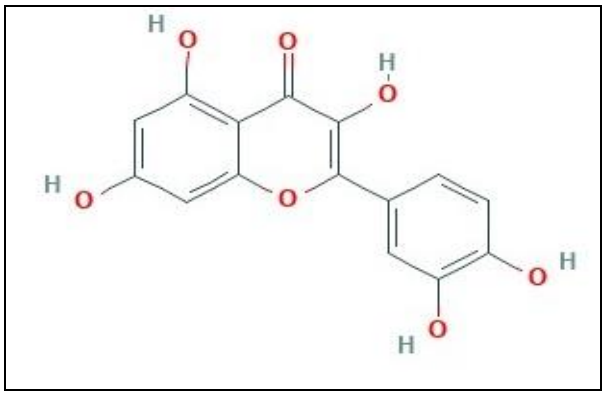

Fig 2

Quercetin is a flavonoid found in many foods and herbs and is a regular component of a normal diet. Extracts of quercetin have been used to treat or prevent diverse conditions including cardiovascular disease, hypercholesterolemia, rheumatic diseases, infections and cancer but have not been shown to be effective in clinical trials for any medical condition. Quercetin as a nutritional supplement is well tolerated and has not been linked to serum enzyme elevations or to episodes of clinically apparent liver injury. The preliminary tests are conducted both for the extract and Quercetin and found to be positive and it is further processed for validation by Spectrophotometry.

Materials and Methods

Preparation of extract

Freshly almond leaves about 250g were washed and soaked in water for 4-5 days. All the extracted solution was filtered and dried powder extract was obtained and further used for tests.

Preparation of reagents

Preparation of test solution: To 100 ml volumetric flask add almond extract powder and make up with distilled water and filtered. Pipette out 10ml of previously prepared solution and dilute to 100ml with diluent to prepare 1000ug/ml concentration. Then diluted with distilled water for futher concentrations.

Preparation of standard solution: Take 0.1g of Quercetin and dilute with 100 distilled water and further diluted to make different concentrations of quercetin to make 25, 50, 75, 100 and 125 ug/ml.

Method Development

Determination of wavelength of maximum absorption:

Different concentrations (25, 50, 75, 100 and 125 ug/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 375nm.

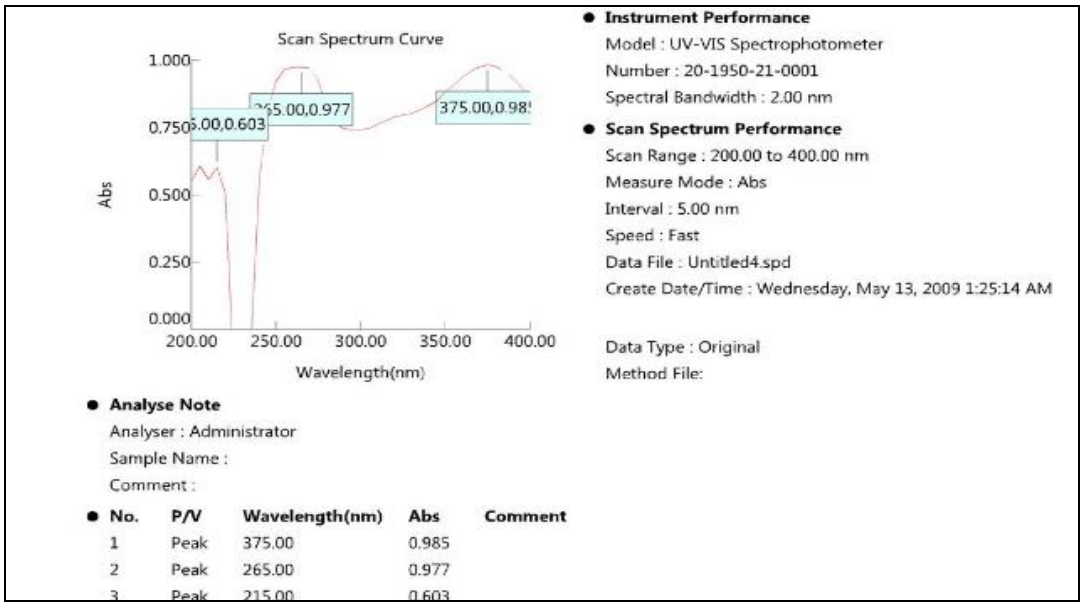

Fig 3: Scanning of Wavelength

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 25µg/mL-125µg/mL solutions. The calibration curves were constructed by plotting absorbance versus concentration and the regression equations were calculated.

Table 1: Calibration curve data of quercetin

S. No	Concentration(µg/ml)	Absorbance
1	0	0
2	25	0.203
3	50	0.428
4	75	0.629
5	100	0.836
6	125	0.986

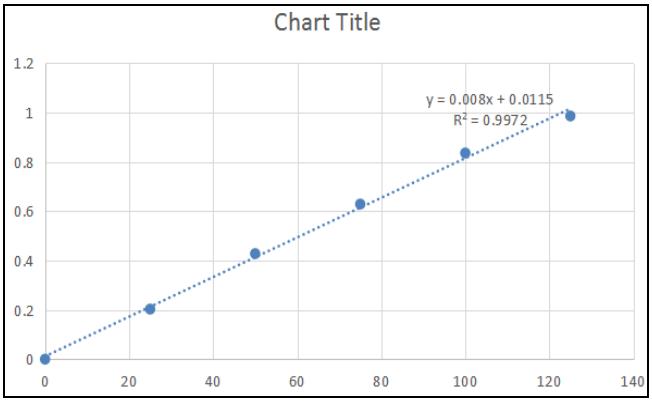

Fig 4: Calibration curve of Quercetin

Precision

Intra-day precision study: Test sample was diluted further to obtain 25-125ug/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	0.962	0.867
2	0.953	0.854
3	0.951	0.848
4	0.953	0.846
5	0.944	0.865
6	0.964	0.864
Mean	0.955	0.853
SD	0.006	0.008
%RSD	0.628	0.938

Accuracy and Recovery Studies: Accuracy of the method was calculated by recovery studies at three different levels (80%, 100% and 120%) 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 almond extract 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
80%	98.64	98.63	0.015	0.015
	98.63			
	98.61			
100%	98.53	98.57	0.032	0.032
	98.58			
	98.59			
120%	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	0.001	0.001
%RSD	0.108	0.109

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 376nm, 377nm and 378nm. The absorbance was measured and assay was calculated for six times.

Table 5: Results of Robustness

S.No	Wave lengths		
	376nm	377nm	378nm
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	0.002	0.004	0.001
%RSD	0.273	0.543	0.136

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:

$LOD = 3.3\ \sigma / S = 0.413\ \mu g/ml$

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

LOQ = 10 σ/S= 1.25 µg /ml

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

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

Table 6: Results of Assay

Drug	Declared Concentration(ug/ml)	Amount found Concentration(ug/ml)	Amount found (%)	%RSD
Sample 1	100	94.5±0.01	94.5±0.01	0.315
Sample 2	100	95±0.01	95±0.01	0.316
Sample 3	100	94±0.01	94±0.01	0.313

IR Interpretation: The functional group identification is made by FTIR analysis and the active components based on the peak value in the region of infrared radiation. The almond leaves extract and Quercetin interpretation is found to be in same and in the nearest range.

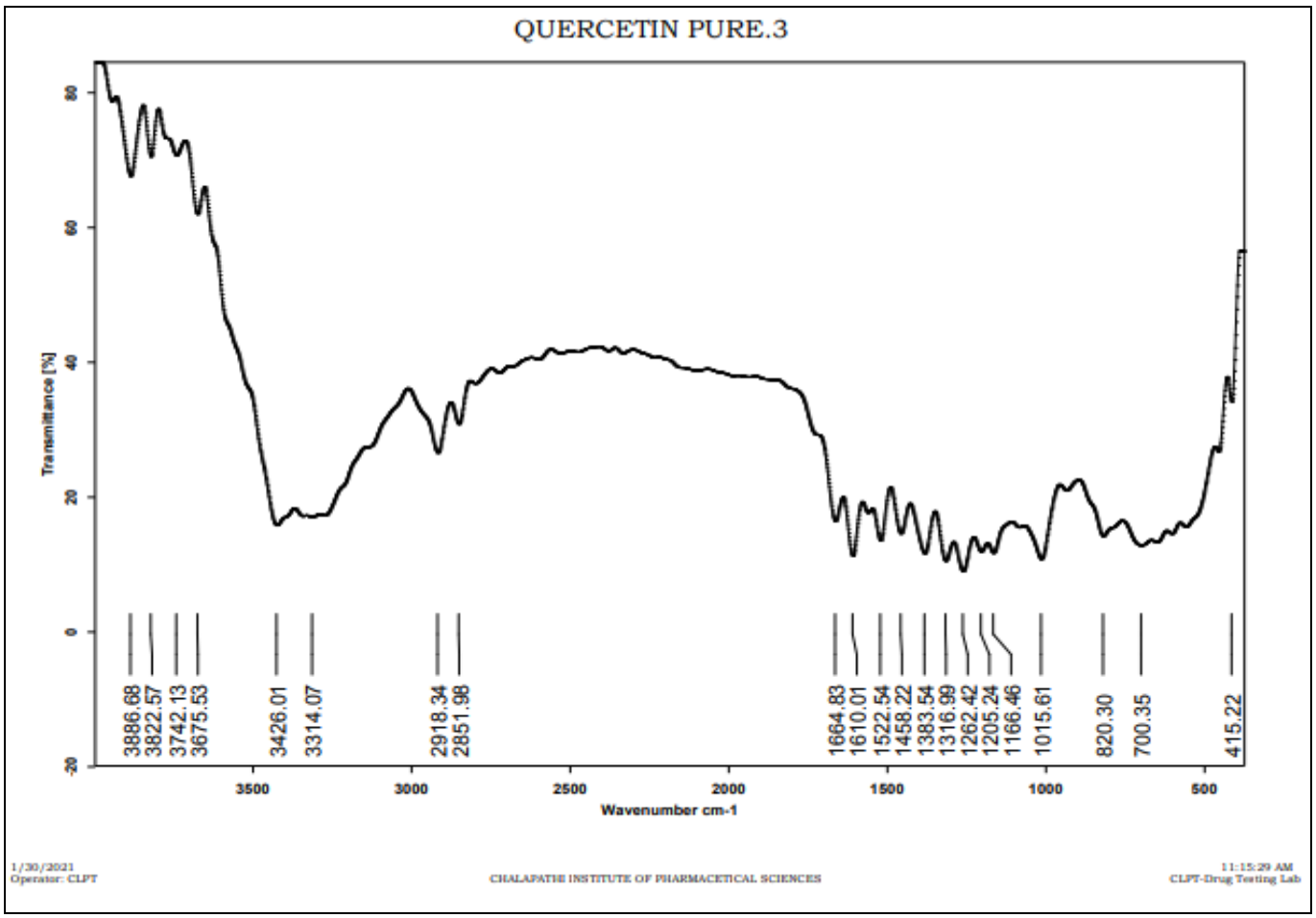

Fig 5: IR Spectrum of Quercetin

Table 7: IR Interpratation Almond extract

Functional group	Peak appearance	Absorption (cm^{-1})
N-H	stretching	3426
C-H	stretching	2918
N-H	Bending	1664
S=O	stretching	1383
C-O	stretching	1262

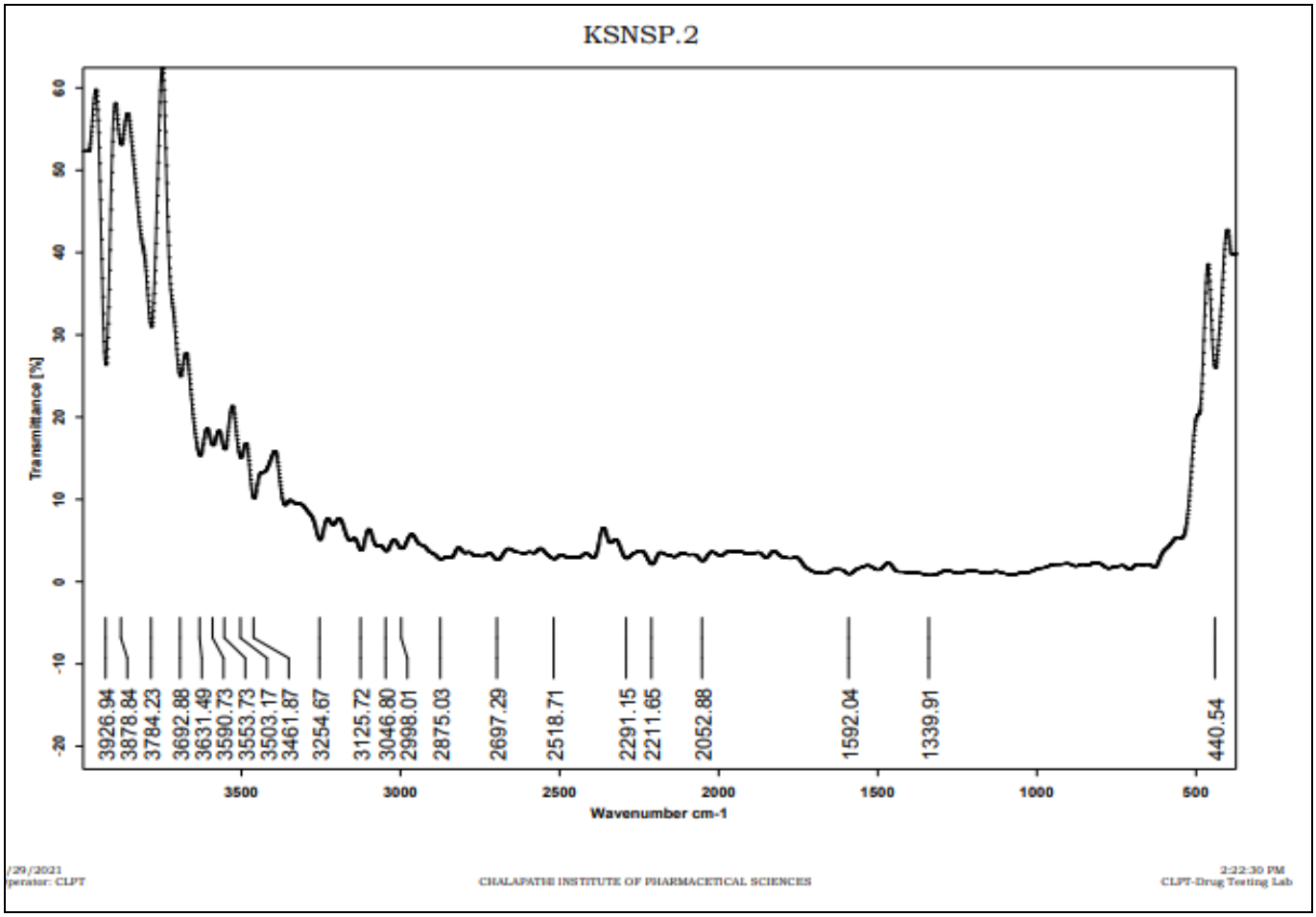

Fig 6: IR Spectrum of almond extract

Table 7: IR Interpratation Almond extract

Functional group	Peak appearance	Absorption (cm^{-1})
S=O	stretching	1399
N-H	bending	1592
N=C=S	stretching	2052
N=C=O	stretching	2291
C-H	stretching	3254

Results and Discussion

The method discussed in the present work provides a convenient and accurate way for analysis of almond leaves extract. The different concentrations were scanned and the wavelength of maximum absorption was found at 375nm. The drug obeyed the Beer's law with the concentration range 25-125ug/ml having line equation y = 0.008x + 0.0115 with correlation coefficient r^2 of 0.9972 and represented excellent linear relationship of the newly developed method. The amount of Quercetin present in almond leaves extract was found to be 0.105ug/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 0.413µg/mL and 1.25 µg/mL respectively. The recovery values for Quercetin present in almond leaves extract 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 0.413 µg/mL and 1.25 µg/mL. The percent relative standard deviation of robustness and ruggedness of the method was 0.136 – 0.543. And the IR interpretation of both almond extracts and quertin were found to have similar functional groups as that of Quercetin pure sample and hence, proposed method was precise, accurate and cost effective. This method could be applicable for quantitative determination of the quercetin present in almond leaves extract.

Acknowledgement

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Conclusion

The different concentrations were scanned and the wavelength of maximum absorption was found at 375nm. The drug obeyed the Beer's law with the concentration range 25-125ug/ml having line equation y = 0.008x + 0.0115 with correlation coefficient r^2 of 0.9972 and represented excellent linear relationship of the newly developed method. The amount of Quercetin present in almond leaves extract was found to be 0.105ug/ml. Finally we conclude that the proposed method was precise, accurate and cost effective. This method could be applicable for

quantitative determination of the Quercetin present in almond leaves extract.

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