

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR ANALYSIS OF FULVIC ACID – A DECOMPOSITION PRODUCT IN SHILAJITH

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Abstract

Fulvic acid is one of the decomposition products of humified material. Humic acid, Fulvic acid, lignin are the most common products of soil humification. The present study was aim to develop a UV method for determining the purity index of Shilajith containing Fulvic acid. The sample extracts were prepared and absorbance was taken at four different wavelengths 280 nm, 260 nm, 472 nm and 664 nm. From the obtained absorbance results, ratio was calculated, where Q1 260/664 , Q2 472/664 , Q3 280/472 shows strongly humified material, humification index and proportion between Lignin and other material respectively. The humification index indicates the presence of Fulvic acid. The result shows good proportion of fulvic acid in the sample when compared with standard Fulvic Acid. The method can be used to analyze the quality of Shilajith in context of Fulvic acid.

Key words: Fulvic acid; Humic acid; Humification; UV spectrophotometer.

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INTRODUCTION

A large number of organic humic products are increasingly being applied worldwide especially in agricultural applications. When decomposition occurs in soil humification takes place which results in production of humic substances. Humic substance can be broken into three components: humic acid (HA), fulvic acid (FA) and insoluble humin mainly contains lignins. It is known that humic and fulvic acids are active components of soil and are important for agriculture and pharmaceutical products.^[1] Fulvic acid is one of the important components of well known Ayurvedic medicine Shilajith. Accurate measurement of these components in commercial products is very important. Several methods including acid precipitation, volumetric method, chromatographic method and spectrophotometric measurement have been established to quantify humic acid and fulvic acid.^[2] The acid precipitation method has been widely accepted for the separation and subsequent quantification of humic acid and Fulvic acid. FAs stimulate plant growth, especially on foliar spraying.

UV/Vis adsorption spectra of HA and FA are featureless over the whole U.V/Vis. wavelength range showing only a strong absorption increase with decreasing wavelength linked to aromatic and other organic chromophores.^{[3][4][5][6]} Evaluation of UV/Vis spectra is either achieved by comparing absorption measurement at a specific wave-length or absorption ratios at two different wavelengths. For characterization / classification of HAs and FAs, a frequently used absorption ratio is $Q2=472/665(E3/E4)$ (absorption at 465 over 665 nm) calculated.^[7] This ratio can presumably be used for estimating the degree of humification. Due to the low absorption at 665 nm, however, the results become strongly influenced by the quality of the background compensation.

In samples of limited concentration, reproducibility suffers under this background compensation problem. Given that the relative shape of HA and FA spectra are similar, any absorption ratios can be used for classification.

The present work is aimed to develop and validate a simple, accurate, rapid and economic method for determination of Fulvic acid in Shilajith.

MATERIALS AND METHOD

Requirement

Ammonia, hydrochloric acid were used as chemicals for the experiment. Standard Fulvic acid was purchased from natural remedies Pvt. ltd. and Shilajith was procured from the local market of Jamnagar.

Sample preparation

1 g sample was taken in 100 ml conical flask. To this weighed sample 50 ml 0.5 N Hydrochloric acid was added. Solution was shaken for 2 h. The solution was allowed to stand for overnight. Next day supernatant or aliquot was collected and absorbance was taken at 280nm, 260 nm, 472 nm and 664 nm.

Standard Fulvic acid solution preparation

The method for preparation of standard solution was same as that of sample solution

Instrumental condition

Instrument: double beam UV spectro-photometer Shimadzu
Working wavelengths: 260 nm, 280 nm, 472 nm, 664 nm
Sample cell: Quartz cuvette
Monochromator: Grating
Detector: Phototube

Procedure

From the standard stock solution aliquot was taken and subjected to UV spectrophotometer. Multi-wavelength absorbance mode parameter was selected in the instrument and the absorbance was read out at selected wavelengths 260 nm, 280 nm, 472 nm and 664 nm. The sample solution was also read out in the same manner as that of standard fulvic acid. Procedure was same for sample solutions. The resultant absorbance values were used for determination of Fulvic acid in Shilajith.

Validation of proposed method - Linearity

From the standard stock solution 1 ml solution was taken in three different test tubes and diluted at 2,4,6 times respectively with water. Absorbance was measured at 4 different working wavelengths taking water as blank. The absorbance values were used to generate linearity graph.

Precision

The precision was done by repeatability. Repeatability was evaluated by measuring the absorbance of sample and standard aliquot solutions at four working wavelengths during the same day at same working condition. For intraday precision measurement, absorbance was taken during 3 different days and for interday, measurement was taken on the same day after every 3 h. Precision (Interday and intraday) was expressed as % relative standard deviation (RSD).

RESULTS AND DISCUSSION

The chemical name of Shilajith is Asphaltum. The amount of active ingredients (fulvic acid) in the raw material varies by the region it is harvested from. Raw Shilajith must be purified and concentrated to increase the level of active ingredients. The proposed method was aimed to develop a method for determination of

Fulvic acid as one of the decomposition product in Shilajith. A simple UV spectrophotometric method was adopted for desired purpose. For measuring absorbance, four different wavelengths 260 nm, 280 nm, 472 nm and 664 nm were selected. Sample and standard aliquot were read out for absorbance values at all selected wavelengths. The results for absorbance are shown in Table 1. To measure fulvic acid, ratio of absorbance was noted where Q1 (269/664) represents non humified material, Q2 (472/664) gives humification index mainly presence of Fulvic acid and Q3 shows proportion between lignin and other humified materials except fulvic acid. (Table 2) Results shows that the Q2 value that is humification index was found to be more as compared to Q1 and Q3 directly indicating the presence of good proportion of fulvic acid in Shilajith. The method was subjected for linearity parameter. Linearity for the Fulvic acid by the proposed method was determined to study its ability to elicit test results which are directly proportional to concentration of the analyte in the sample. The response was found to be linear over the three dilution range (2, 4 and 6 times). The correlation coefficient was 0.986. (Table 3 & Figure 1) The sample was also studied for interday and intraday precision, the result of the solution at medium concentration is presented in Table 4. The intraday precision was determined by replicate injection of standard and sample solution by an interval of 3 hrs on the same day. From the results, the % RSD of absorbance for Shilajith sample and fulvic acid was calculated and found to be less than 2 % which was within the acceptance criteria for system precision. The interday precision of the method was determined by performing the assay on different days to check the reproducibility. The test result was found to be satisfactory with respect to relative standard deviation (%RSD). Hence the proposed method was found to provide high degree of precision and reproducibility. (Table 3)

Table 1: Absorbance values for Sample and standard solutions

S.No	Sample	Absorbance			
		E1=260 nm	E2=280 nm	E3=472 nm	E4=664 nm
1	Fulvic acid	-0.306	-0.220	0.130	0.034
2	Shilajith	-0.289	-0.238	0.075	0.027

Table 2: Ratio of absorbance values for samples and stand solution

S.No.	Sample	Absorbance ratio index		
		Q1=260/664(E1/E4)	Q2=472/664(E3/E4)	Q3=280/472(E2/E3)
1	Fulvic acid	-9.0	3.8	-1.6
2	Shilajith	-10.7	2.7	-5.2

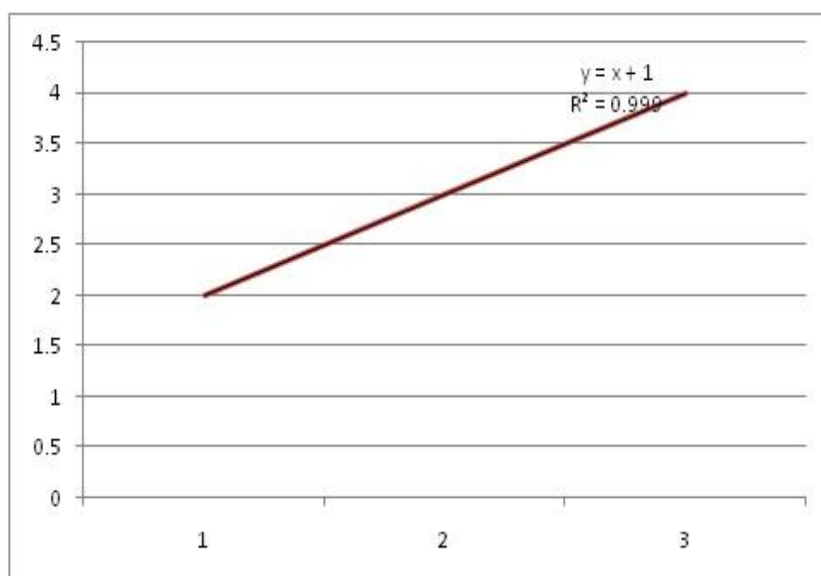
Table 3: Results for linearity of Fulvic Acid

Concentration	Absorbance(Q ₂)	Co-relation coefficient
Double dilution	1.83	0.999
Triple dilution	1.09	
Quadruple dilution	0.95	

Table 4: Results for precision For Fulvic acid

Precision	% RSD Fulvic acid	% RSD Shilajith
a) Interday	0.98	0.12
b) Intraday	0.95	0.99

Figure 1: Linearity graph Fulvic acid



CONCLUSION

The purity index can be used as one of the testing parameter to determine the quality of Shilajith. The developed UV Spectrophotometric method is a precise, specific, reliable, fast and economic for the determination of Fulvic acid in decomposed material.

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