International Journal of Recent Advances in Multidisciplinary Topics
Volume 2, Issue 11, November 2021
https://www.ijramt.com | ISSN (Online): 2582-7839

Determination of Humic Acid and Fulvic Acid by Spectrophotometric Analysis from the Effervescent Tablet

Ashpak Inamdar[1*], Mahendra Godase[2], Ameya Mulay[3], Vilas Mhaske[4]
[1,2,3,4]*Sci Tech Specialities Private Limited, Nashik, India*

***Abstract*: This humic Acid and Fulvic Acid substances are applicable in agricultural, environmental, biomedicine. UV Spectrophotometric Analysis of the organic compounds like fulvic acid, humic acid. Humic acid (HA) represents the organic material most widespread in nature and have positive effects on plant physiology influencing nutrient uptake and root architecture. These are representing the most variations in chemical, functional groups and spectroscopic measurements were observed among the extracted humic and fulvic acids. More humic substances were extracted from the normal soil than other soil types, with the majority being humic acid. Fulvic acid extracted from all soils contained mostly aliphatic, O-substituted alkyl, and carboxylic groups and small amounts of carbonyl groups. Based on the spectroscopic analysis, no significant differences were detected among different types of humic substances. The aim of our work was to the application of UV-Vis spectrophotometry method to Humic acid determination after its extraction by Sodium pyrophosphate alkali solvent and fulvic acid extracted by layer separation technique. The analytical validation parameter of this method were evaluated. The results for the proposed method are comparable or more applied than them. Also this methodology is easier and faster than others methods.**

***Keywords*: fulvic acid, humic acid, uv/vis spectrophotometry.**

1. Introduction

Their origin The term humus dates back to the time of the Romans, when it was used to designate the soil as a whole. Many of the procedures he developed for the preparation of humic acids became generally adopted, such as pretreatment of the soil with dilute mineral acids prior to the extraction with alkali. Humic acid is the dark-coloured organic material which can be extracted from soil by various reagents and which is insoluble in dilute acid. Fulvic acid is the brown coloured material which remains in solution after removal of humic acid by acidification.

That fraction of humic substances that is not soluble in water under acid conditions (below pH 2) but becomes soluble at greater pH. That fraction of fulvic acid substances that is soluble under all pH conditions.

Humic acid: Since the dawn of modern chemistry, humic substances are among the most studied among the natural materials. Despite long study, their molecular structure and chemical remains elusive. The traditional view is that humic substances are heteropoly condensates, in varying associations with clay. A more recent view is that relatively small molecules also play a role. Humic substances account for 50 – 90% of cation exchange capacity. Similar to clay, char and colloidal humus hold cation nutrients. Humic matter in isolation is the result of a chemical extraction from the soil organic matter or the dissolved organic matter and represent the humic molecules distributed in the soil or water. A new understanding views humic substances not as high-molecular-weight macropolymers but as heterogeneous and relatively small molecular components of the soil organic matter auto-assembled in supramolecular associations and composed of a variety of compounds of biological origin and synthesized by abiotic and biotic reactions in soil.

Fulvic acid: Fulvic acids are a family of organic acids, natural compounds, and components of the humus (which is a fraction of soil organic matter). [9] The small molecular weight fulvic acids remain in solution after precipitation of the high molecular weight humic acids by acidification at pH = 1. Fulvic acids are produced by microbial degradation of plant matter in a soil with sufficient oxygen. This organic matter is soluble in strong acid (pH = 1).

2. Introduction

Humic acid:

List of glassware required for the test:

Sr. No.	Glassware	Capacity	Required quantity
1	Beaker	100 ml	04 Nos
2	Pipette	10 ml	02 Nos
3	Volumetric flask	100 ml	05 Nos

List of instruments required for the test:

1) Analytical Balance (Capacity -220 gm, Least Count- 0.0001gm)
2) UV Spectrophotometer
3) Sonicator
4) Mortar pestle
5) Water bath

List of Chemicals and material required for the test:

1) Sodium Hydroxide

*Corresponding author: aspakinamdar786@gmail.com

2) Tetra sodium Pyrophosphate anhydrous

Solvent Preparation (A):

Weight 15 gm of Tetra Sodium Pyrophosphate anhydrous (TSPP anhydrous) and 7gm of Sodium Hydroxide in 1000 ml volumetric flask and dissolve in purified water sonicate and shake to produce clear solution. Make a volume up to 1000 ml with purified water.

Standard Stock solution preparation:

Weigh 100 mg of Humic Acid in 100 ml volumetric flask, add 50 ml above solvent (A) with continuous shaking for 5 min and make up volume by using same solvent (A) and sonicate for 5 min.

Take 10 ml of standard stock solution and 50 ml of solvent (A) in 100ml volumetric flask, shake and make up the volume with same solvent (A).

Sample Stock solution preparation:

Weigh and make a fine powder of 3 tablets in mortar pestle. Weigh accurately a quantity equivalent to 100 mg of humic acid in 100 ml volumetric flask, add 50 ml of solvent (A) with continuous shaking for 5 min Make up the volume upto the mark with same solvent (A) and sonicate for 5 min ensuring the complete dissolution of blend.

Take 10 ml of the sample stock solution and 50 ml with solvent (A) in 100 ml volumetric flask. Shake and make up volume by using same solvent (A) and sonicate for 5 min.

Procedure:

Place both Standard and Sample in water bath for at 900 C exactly about 90 min. Shake both the standard and sample solution in every 30 min interval for complete reaction. After it cool both flask and take absorbance at 465nm against solvent (A) as a blank.

Validation Parameters:

1) Suitability

Procedure: Prepared the standard solution as per method and take absorbance at 465 nm of the same solution at 5 different times.

Sr.no.	Sample	Absorbance at WL 465nm
1	Standard 1	0.868
2	Standard 2	0.871
3	Standard 3	0.870
4	Standard 4	0.869
5	Standard 5	0.872
	AVG.	**0.870**
	Std. Deviation	**0.001581**
	%RSD	**0.1817 %**

Acceptance Criteria:

% RSD should NMT 2% for 5 Replicates of standard solution.

Conclusion:

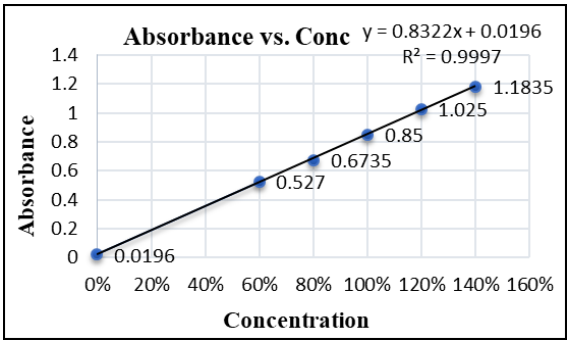
From the above results the %RSD is 0.2260 % which is not more than the2%, so passes the System Suitability criteria.

2) Linearity

Procedure:

Prepared the sample solutions of different concentration of 60%, 80%, 100%, 120% and 140% and measure the absorbance of each concentration. Plot the graph of concentration vs. Absorbance and calculate correlation coefficient (R^2).

Sample	Sample Conc.	Sample Abs.	Avg. Abs.
Sample 1	60%	0.528	0.527
	60%	0.526	
Sample 2	80%	0.673	0.6735
	80%	0.673	
Sample 3	100%	0.849	0.850
	100%	0.851	
Sample 4	120%	1.023	1.025
	120%	1.025	
Sample 5	140%	1.183	1.1835
	140%	1.184	



Acceptance criteria:

1. Correlation coefficient should be NLT 0.99

Conclusion:

From the graphical representation correlation coefficient was found to be satisfactory i.e. 0.999.

From the above results obtained, it is concluded that concentration is directly proportional to the absorbance for given method on the given set of conditions.

3) Specificity

Prepared a set of 6 different samples with spiking different concentrations of placebo. This process ensures the identity of analyte in a designed formulation.

STD	STD OD	SPL NAME	SPL OD	Samples
STD-1	0.868	SPL-1	0.865	Sample without spike
STD-2	0.869	SPL-2	0.861	Sample with 10 % placebo spike
STD-3	0.867	SPL-3	0.868	Sample with 20 % placebo spike
STD-4	0.868	SPL-4	0.864	Sample with 30 % placebo spike
STD-5	0.867	SPL-5	0.862	Sample with 40 % placebo spike
		SPL-6	0.870	Sample with 50 % placebo spike
		SPL-6	0.008	placebo spike without sample
AVG	0.8678			
STDEV	0.000837			
%RSD	0.096412			

Sample	% Assay of Humic Acid
Sample Without Spike	101.18
Sample with 10% placebo spike	100.71
Sample with 20% placebo spike	100.55
Sample with 30% placebo spike	102.05
Sample with 40% placebo spike	100.82
Sample with 50% placebo spike	100.78
Average	101.01
Standard deviation	0.60533
% RSD	0.59925

Acceptance Criteria: The %RSD of assay values of all the samples should NMT 2.0.

Conclusion:

From the above data, it is observed that there is no any interference from any of the concentration of the placebo materials on assay of the active materials; hence this proves unequivocally of the analyte in formulation.

4) Accuracy and % Recovery

Procedure:

Prepared four samples with different concentrations like 80%, 100% and 120% and analyze the samples as per analytical method.

Sr.no.	SPL-1 80%	SPL-2 100%	SPL-3 120%
Limit	98-102%	98-102%	98-102%
1	100.62	100.81	100.52
2	101.08	100.10	100.03
3	100.77	100.58	100.54
Mean	**100.82**	**100.50**	**100.36**
Std Dev	**0.235**	**0.364**	**0.291**
%RSD	**0.233%**	**0.363%**	**0.290%**

Standard	Std. Abs	Sample Name	Sample Wt.	Sample Abs.
Std-1	0.836	Spl-1(80%)	0.220	0.694
Std-2	0.836	Spl-1(80%)	0.219	0.694
Std-3	0.835	Spl-1(80%)	0.220	0.695
Std-4	0.836	Spl-2 (100%)	0.274	0.866
Std-5	0.836	Spl-2 (100%)	0.275	0.863
		Spl-2(100%)	0.274	0.864
Avg. (Standard)	0.868	Spl-3 (120%)	0.330	1.040
Std Dev	0.001	Spl-3(120%)	0.331	1.038
RSD (%)	0.096	Spl-3(120%)	0.329	1.037

Sample	Assay (%)
Spl-1	100.62
Spl-1	101.08
Spl-1	100.77
Spl-2	100.81
Spl-2	100.10
Spl-2	100.58
Spl-3	100.52
Spl-3	100.03
Spl-3	100.54
Avg.	100.56
Std. Dev	0.331
RSD	0.330

Acceptance criteria:

% Recovery for each stage should be between 98 -102 %.

Conclusion: As per data sheet, all assay values are within specified limit, which indicates that the given method is accurate for the analysis of Humic acid.

5) Precision

Prepare single standard& 6 different samples from a uniformly mixed blend.

Standard	Standard OD	Sample Name	Sample Weight	Sample OD
Std-1	0.867	Spl-1	0.276	0.872
Std-2	0.868	Spl-2	0.276	0.871
Std-3	0.868	Spl-3	0.275	0.873
Std-4	0.869	Spl-4	0.276	0.872
Std-5	0.872	Spl-5	0.278	0.875
		Spl-6	0.278	0.872
Avg.	0.868			
Std. Dev	0.001			
RSD	0.081 %			

Sample	Assay (%)
Spl-1	100.75
Spl-2	100.64
Spl-3	101.24
Spl-4	100.75
Spl-5	100.37
Spl-6	100.03
Avg.	100.75
Std. Dev	0.313
(%) RSD	0.310 %

Acceptance criteria:

% Assay: The % RSD of all assay values should NMT 2.0 %

Conclusion:

As per acceptance criteria the %RSD of assay samples 0.310 which is below the limit 2.0 %.

From above results, it is concluded that the precision study for a given method is found satisfactory.

6) Solution Stability

Procedure:

The solution stability parameter was verified by reading the absorbance of prepared standard and sample solutions. Read the absorbance at different time intervals.

Sr. no.	Time	Std Solution	Spl 01 Solution	Spl 02 Solution
1)	Initial	0.868	0.867	0.871
2)	1Hr	0.865	0.866	0.870
3)	2Hr	0.866	0.867	0.872
4)	3Hr	0.864	0.867	0.871
5)	4Hr	0.865	0.866	0.871
Average		**0.866**	**0.867**	**0.871**
Std. Dev		**0.001**	**0.001**	**0.001**
% RSD		**0.094**	**0.067**	**0.094**

Time	% Difference Std. sol	% Difference Spl. sol 01	% Difference Spl. sol 02
Initial			
1Hr	**-0.35%**	**-0.12%**	**-0.11%**
2Hr	**-0.23%**	**0.00%**	**0.11%**
3Hr	**-0.46%**	**0.00%**	**0.00%**
4Hr	**-0.35%**	**0.12%**	**0.00%**

Acceptance Criteria:

% RSD of standard readings should NMT2.0%.

% RSD of sample readings should NMT 2.0 %.

% Difference should NMT± 2.0%.

Formula: (Absorbance after interval - Initial Absorbance)/ Initial Absorbance *100

Conclusion:

As from the above sample absorbance readings, both standard and sample solution was stable for 4 hours.

7) Robustness

Procedure:

We are analyzing the sample for different heating time.

Different heating time: Prepare standard and sample solution in duplicate as per analytical procedure. Change in the heating time. (i.e. normal heating time is 90 min so) it should be 85.0 min and 95 min respectively.

	Standard Absorbance	Sample 1 Absorbance	Sample 2 Absorbance
85 min	0.867	0.875	0.876
90 min	0.866	0.873	0.875
95 min	0.863	0.875	0.875
Avg.	0.865	0.874	0.875
Stdev.	0.0021	0.0012	0.0006
%RSD	0.2406	0.1321	0.0660

Acceptance criteria:

1. % RSD of assay of sample for all heating time should be NMT 2.0 %.
2. Assay of each sample is within 98-102 %.

8) Ruggedness

A) Different Analysts on same day

Procedure:

Prepare 3 Samples as per proposed method of analysis and analyze it.

	Analyst 1		Analyst 2	
	Observed Absorbance	% Assay	Observed Absorbance	% Assay
Standard	**0.865**		**0.869**	
Sample 1	0.871	100.62	0.876	100.01
Sample 2	0.870	100.87	0.863	100.33
Sample 3	0.867	100.26	0.872	100.64
Mean		**100.92**		**100.32**
Std. Dev		**0.320**		**0.313**
% RSD		**0.317**		**0.312**
Mean % Assay	**100.92**		**100.32**	
Mean % RSD of assay	**0.416**			

B) Same analyst on different day

Procedure:

Prepare 3 Samples as per proposed method of analysis and analyze it.

	Day 1		Day 2	
	Observed Absorbance	% Assay	Observed Absorbance	% Assay
Standards	**0.862**		**0.866**	
1	0.868	100.26	0.8964	100.79
2	0.865	100.37	0.865	100.54
3	0.869	100.74	0.867	100.04
Mean		**100.79**		**100.46**
Std Dev.		**0.558**		**0.0.379**
% RSD		**0.553**		**0.378**
Mean % Assay	**=100.79 %**		**=100.46%**	
Mean % RSD of assay	**0.235 %**			

Acceptance Criteria:

1) % RSD of % assay of both the analysts should not NMT 2%.
2) % RSD (Intraday) of all assay values of two different analysts should not be more than 2.0 %.

Fulvic Acid:

List of glassware required for the test:

Sr. No.	Glassware	Capacity	Required quantity
1.	Beaker	100 ml	03 Nos
2.	Pipette	10 ml	02 Nos
3	Volumetric flask	100 ml	05 Nos
4	Funnel	N.A	02 Nos

List of instruments required for the test:

1) Analytical Balance (Capacity -220gm, Least Count-0.0001gm)
2) UV Spectrophotometer
3) Magnetic stirrer
4) Sonicator
5) pH Meter
6) Mortar pestle

List of Chemicals and material required for the test:

1) Sodium Hydroxide
2) Conc. Hydrochloric acid

Solution preparation:

0.1 N Sodium hydroxide: Take 4.2 gm of sodium hydroxide in 1000 ml volumetric flask and dilute up to the mark by using purified water.

Standard Preparation:

Weigh accurately 100 mg of fulvic acid working standard in 100 ml volumetric flask make up with 0.1N NaOH, shake flask and stirred on magnetic stirrer for 30 min. Adjust the pH 1 with Conc. HCl Shake and constant kept the flask a side for separation of layers up to 1 hrs. One of upper layer is clear and another lower layer is black precipitate. Pipette out 20ml clear upper layer solution and filter through filter paper. Take 10ml of filtrate in 100ml volumetric flask and makeup the volume with 0.1N NaOH.

Sample Preparation:

Weigh and make a fine powder of 3 tablets in mortar pestle. Weigh accurately a quantity equivalently to 100 mg of fulvic acid in 100 ml volumetric flask and make up volume with 0.1N NaOH, shake flask and stirred the sample on magnetic stirrer for 30 min. Adjust the pH 1 with Conc. HCl, Shake and constant kept the flask a side for separation of layers up to 1 hr. One of upper layer is clear and another lower layer is black precipitate. Pipette out 20ml clear upper layer solution and filter through filter paper. Take 10ml of filtrate in 100ml volumetric flask and makeup the volume with 0.1N NaOH.

Blank Preparation:

Take 50 ml of 0.1N NaOH and adjust the pH 1 with Conc. HCl and used as a blank.

Take absorbance at 280 nm against blank.

1) System suitability

Sr.no.	Sample	Absorbance at WL 280
1	Standard 1	0.343
2	Standard 2	0.351
3	Standard 3	0.351
4	Standard 4	0.354
5	Standard 5	0.347
	AVG.	**0.3492**
	Std. Deviation	**0.004266**
	% RSD	**1.222%**

Acceptance Criteria:

% RSD should NMT 2% for 5 Replicates of standard solution.

Conclusion:

From the above results the %RSD is 1.222% which is not more than the2%, so passes the System Suitability criteria.

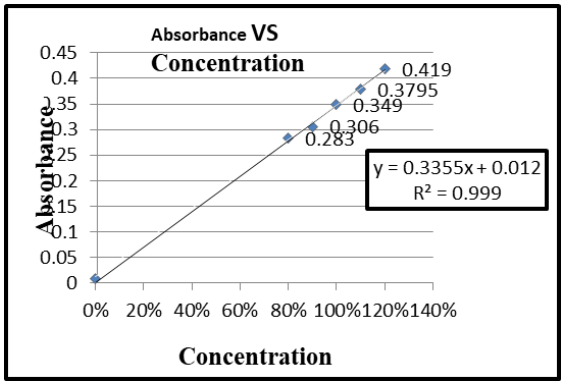
2) Linearity

Procedure:

Prepared the sample solutions of different concentration of 80%, 90%, 100%, 110% and120% and measure the absorbance of each concentration.

Plot the graph of concentration Vs Absorbance and calculate correlation coefficient (R^2).

Sample	Sample Concentration	Sample Absorbance	Avg. Absorbance
Sample 1	80%	0.282	0.283
	80%	0.284	
Sample 2	90%	0.305	0.306
	90%	0.307	
Sample 3	100%	0.346	0.349
	100%	0.352	
Sample 4	110%	0.381	0.380
	110%	0.378	
Sample 5	120%	0.419	0.419
	120%	0.419	



Acceptance criteria:

Correlation coefficient should be NLT 0.99

Conclusion:

From the graphical representation correlation coefficient was found to be satisfactory i.e. 0.999

From the above results obtained, it is concluded that concentration is directly proportional to the absorbance for given method on the given set of conditions.

3) Specificity

Procedure: Prepared a set of 6 different samples with spiking different concentrations of placebo. This process ensures the identity of analyte in a designed formulation.

Acceptance Criteria: The %RSD of assay values of all the samples should NMT 2.0.

Conclusion:

From the above data, it is observed that there is no any interference from any of the concentration of the placebo materials on assay of the active materials; hence this proves unequivocally of the analyte in formulation.

Sample	% Assay of Fulvic Acid
Sample without Spike	100.24
Sample with 10% placebo spike	100.72
Sample with 20% placebo spike	100.36
Sample with 30% placebo spike	100.23
Sample with 40% placebo spike	100.59
Sample with 50% placebo spike	100.46
Average	100.43%
Standard deviation	0.218
%RSD	0.217%

STD	STD Absorbance	Spl Absorbance	Spl Wt	Spl Absorbance	Samples
STD-1	0.355	SPL-1	0.1045	0.352	Sample without spike
STD-2	0.349	SPL-2	0.1046	0.354	Sample with 10 % placebo spike
STD-3	0.351	SPL-3	0.1026	0.346	Sample with 20 % placebo spike
STD-4	0.352	SPL-4	0.1063	0.358	Sample with 30 % placebo spike
STD-5	0.346	SPL-5	0.1068	0.361	Sample with 40 % placebo spike
		SPL-6	0.1022	0.345	Sample with 50 % placebo spike
		SPL-7	0.1013	0.008	Placebo spike without sample
AVG	0.3506				
STDEV	**0.0034**				
% RSD	**0.96%**				

From the above data, it is observed that there is no any interference from any of the concentration of the placebo materials on assay of the active materials; hence this proves unequivocally of the analyte in formulation.

4) Accuracy and % Recovery

Procedure:

Prepared three samples with different concentrations like 80%, 100% and 120% and analyze the samples as per analytical method.

Standard	Standard Absorbance	Sample Name	Sample Wt.	Sample Absorbance
Std-1	0.315	Spl-1(80%)	0.6278	0.253
Std-2	0.315	Spl-1(80%)	0.6278	0.251
Std-3	0.314	Spl-1(80%)	0.6278	0.252
Std-4	0.314	Spl-2(100%)	0.7815	0.308
Std-5	0.312	Spl-2(100%)	0.7815	0.309
		SPL-2(100%)	0.7815	0.313
Avg.	**0.314**	Spl-3(120%)	0.9986	0.376
Std. Dev	**0.001**	Spl-3(120%)	0.9986	0.373
RSD (%)	**0.390%**	Spl-3(120%)	0.9986	0.374

Sr.no.	SPL-1 80%	SPL-2 100%	SPL-3 120%
Limit	98-102%	98-102%	98-102%
1	100.04	100.55	100.09
2	100.15	100.69	100.20
3	100.32	100.50	100.93
Mean	**100.17**	**100.58**	**100.41**
Std. Dev	**0.142**	**0.096**	**0.457**
%RSD	**0.141**	**0.026**	**0.456**

Sample	Assay (%)
Spl-1	100.04
Spl-1	100.15
Spl-1	100.32
Spl-2	100.55
Spl-2	100.69
Spl-2	100.50
Spl-3	100.09
Spl-3	100.20
Spl-3	100.93

Acceptance criteria:

% Recovery for each stage should be between 98 -102 %

Conclusion:

As per data sheet, all assay values are within specified limit, which indicates that the given method is accurate for the analysis of Fulvic Acid.

5) Precision

Procedure:

Prepare single standard and 6 different samples from a uniformly mixed blend.

Standard	Standard Absorbance	Sample Name	Sample Weight	Sample Absorbance
Std-1	0.348	Spl-1	0.573	0.363
Std-2	0.348	Spl-2	0.577	0.365
Std-3	0.348	Spl-3	0.566	0.355
Std-4	0.347	Spl-4	0.573	0.361
Std-5	0.347	Spl-5	0.574	0.362
		Spl-6	0.572	0.361
Avg.	**0.348**			
Std. Dev	**0.001**			
RSD	**0.158 %**			

Sample	Assay (%)
Spl-1	100.69 %
Spl-2	100.37 %
Spl-3	99.69 %
Spl-4	100.14 %
Spl-5	100.24 %
Spl-6	100.31 %
Avg.	**100.23%**
Std. Dev	**0.365**
(%) RSD	**0.364 %**

Acceptance criteria:

% Assay: The % RSD of all assay values should NMT 2.0 %

Conclusion:

As per acceptance criteria the %RSD of assay samples 0.364 which is below the limit 2.0 %.

From above results, it is concluded that the precision study for a given method is found satisfactory.

6) Solution Stability

Procedure:

The solution stability parameter was verified by reading the absorbance of prepared standard and sample solutions. Read the absorbance at different time intervals.

Sr.no.	Time	Std Solution	Spl 01 Solution	Spl 02 Solution
1)	Initial	0.349	0.355	0.353
2)	1Hr	0.347	0.353	0.350
3)	2Hr	0.349	0.351	0.353
4)	3Hr	0.348	0.352	0.354
5)	4Hr	0.356	0.356	0.352
Average		**0.348**	**0.353**	**0.352**
Std. Dev		**0.001**	**0.002**	**0.002**
% RSD		**0.275**	**0.611**	**0.485**

Time	% Difference Std sol	% Difference Spl sol 01	% Difference Spl sol 02
Initial			
1Hr	**-0.57%**	**-0.56%**	**-0.85%**
2Hr	**0.00%**	**-1.13%**	**0.00%**
3Hr	**-0.29%**	**-0.85%**	**0.28%**
4Hr	**-0.57%**	**0.28%**	**-0.28%**

Acceptance Criteria:

% RSD of standard readings should NMT2.0%.

% RSD of sample readings should NMT 2.0 %.

% Difference should NMT± 2.0%.

Formula: (Absorbance after interval - Initial Absorbance)/ Initial Absorbance *100

Conclusion:

As from the above sample absorbance readings, both standard and sample solution was stable for 4 hours.

7) Robustness

Procedure:

We are analyzing the sample for different stirring time.

Different stirring time: Prepare standard and sample solution in duplicate as per analytical procedure. Change in the stirring time. (i.e. normal stirring time is 30 min so) it should be 25.0 min and 35 min respectively.

	Standard Absorbance	Sample 1 Absorbance	Sample 2 Absorbance
25 min	0.353	0.361	0.359
30 min	0.351	0.357	0.355
35 min	0.352	0.359	0.357
Avg.	0.352	0.359	0.357
Stdev.	0.112	0.347	0.111
% RSD	0.111	0.346	0.111

Acceptance criteria:

1. % RSD of assay of sample for all stirring time should be NMT 2.0 %.
2. Assay of each sample is within 98-102 %.

8) Ruggedness

A) Different analysts on same day

Procedure:

Prepare 3 Samples as per proposed method of analysis and analyze it.

	Analyst 1		Analyst 2	
	Observed Absorbance	**% Assay**	**Observed Absorbance**	**% Assay**
Standards	**0.351**		**0.351**	
1	0.358	100.82%	0.359	100.19%
2	0.360	100.83%	0.357	99.99%
3	0.356	100.25%	0.359	100.37%
Mean		**100.63%**		**100.19**
Std. Dev.		**0.335**		**0.191**
% RSD		**0.333%**		**0.191 %**
Mean % Assay	**=100.63%**		**=100.41%**	
Mean % RSD of assay	**0.316 %**			

B) Same analyst on different day

Procedure:

Prepare 3 Samples as per proposed method of analysis and analyze it.

	Day 1		Day 2	
	Observed Absorbance	**% Assay**	**Observed Absorbance**	**% Assay**
Standards	**0.345**		**0.351**	
1	0.559	100.27%	0.358	100.45%
2	0.553	100.78%	0.390	99.47%
3	0.555	100.70%	0.358	100.09%
Mean		**100.58%**		**100.34**
Std. Dev.		**0.275**		**0.213**
% RSD		**0.273%**		**0.213 %**
Mean % Assay	**=100.58%**		**=100.46%**	
Mean % RSD of assay	**0.176 %**			

Acceptance Criteria:

1. % RSD of % assay of both the analysts should not NMT 2%.
2. % RSD (Intraday) of all assay values of two different analysts should not be more than 2.0 %.

3. Conclusion

This paper presented an overview on determination of humic acid and fulvic acid by spectrophotometric analysis from the effervescent tablet.

References

[1] Stevenson, F.J. (1982) Humus Chemistry, Wiley, New York.

[2] Aiken, G.R., McKnight, D.M., Wershaw, R.L. and Mac Carthy, P. (1985) Humic Substances in Soil, Sediment and Water. Geochemistry, Isolation and Characterization. Wiley, New York.

[3] Ponomarenko, E.V.; Anderson, D.W. (2001), "Importance of charred organic matter in Black Chernozem soils of Saskatchewan", Canadian Journal of Soil Science, 81 (3): 285–297.

[4] Piccolo, A. (2002). The Supramolecular structure of humic substances. A novel understanding of humus chemistry and implications in soil science. Advances in Agronomy. 75. pp. 57–134.

[5] Weil, Ray R.; Brady, Nyle C. (2016). The Nature and Properties of Soils (15th ed.). Columbus: Pearson (published April 11, 2016). p. 554.

[6] Piccolo A. (2016). "In memeoriam of F. J. Stevenson and the Question of humic substances". Chemical and Biological Technologies in Agriculture.

[7] Drosos M.; et al. (May 15, 2017). "A molecular zoom into soil Humeome by a direct sequential chemical fractionation of soil". The Science of the Total Environment. 586: 807–816.

[8] Piccolo A.; et al. (2018). "The Molecular Composition of Humus Carbon: Recalcitrance and Reactivity in Soils". The Molecular Composition of Humus Carbon: Recalcitrance and Reactivity in Soils. In: The Future of Soil Carbon, Wiley and Sons. pp. 87–124.

[9] Bremner, J. M. (January 1951). "A Review of Recent Work on Soil Organic Matter Part I". Journal of Soil Science. 2 (1): 67–82.

[10] Aiken, G.R.; McKnight, D.M.; Thorn, K.A.; Thurman, E.M. (July 1992). "Isolation of hydrophilic organic acids from water using nonionic macroporous resins". Organic Geochemistry. 18 (4): 567–573.

[11] Chefetz, Benny; Chen, Yona; Hadar, Yitzhak; Hatcher, Patrick (1998-03-01). "Characterization of Dissolved Organic Matter Extracted from Composted Municipal Solid Waste". Soil Science Society of America Journal. 62 (2): 326 –332.

[12] Schnitzer, M. (1977). "Recent findings on the characterization of humic substances extracted from soils from widely differing climatic zones" Proceedings of the Symposium on Soil Organic Matter Studies. Environment with adeequate oxygen. Braunsweig: 117–131.

[13] ICH, Validation of analytical procedures: Text and methodology, International conference on harmonization, IFPMA, Geneva, 1996.