

# Analytical method validation and determination of New HPLC Method for Oxalic acid in Acacia Nilotica.

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## Abstract:

The present work is concerned with application of simple Isocratic HPLC method for determination of (Oxalic acid) in Acacia Nilotica tree.

The method was developed and validated according to ICH guideline. The validated method was linear in the ranged of 0.1 mg/ml to 0.4 mg/ml with  $R^2$  0.9999, Accuracy recovered was 99.0%, System suitability was assessed by  $RSD\% = 0.29\%$ , the Repeatability was assessed by  $RSD\% = 0.35\%$ , intermediated precision was assessed by  $RSD\% 1.4\%$ .

## Introduction:

Many plants are still believed to be a main source of traditional medicine in Sudan, although modern medicine is being practiced widely in even very nomadic life where traditional treatment is dominant. However Acacia nilotica tree which grows spontaneously almost all over the country is considered the most important plant used by Sudanese people for the treatment of chest infections, sore throat and cold.

Acacia nilotica (L.) Delile belongs to the Fabaceae family and the subfamily Mimosoideae; and commonly called Gum arabic tree. As the young pods and seeds are eaten roasted by the people in South Iran. ( Abbasian, K., Ziarati, P. and Asgarpanah, J. (2016))

## MATERIALS AND METHODS Chemicals:

**Reagents:** Potassium dihydrogen phosphate (AR) (Thomas baker), water (water for injection) phosphoric acid (AR) (Merck), Oxalic acid (Fisher).

**Instruments:** Weighing Balance Mettler Toledo JS1603C/A, Sonicator Ultrasonic Elma Elmasonic P300H. PH Meter Mettler Toledo, Filter Paper 0.2  $\mu\text{m}$ . syringe filter 0.45  $\mu\text{m}$ , HPLC Waters Model: Alliance 2695.

### **Chromatographic parameters:**

\*Temperature: 10°C.

\*Flowrate: 0.7 ml/min.

\*Mobile phase: add 0.68% potassium dihydrogen phosphate buffer (PH=2.8 adjusted by phosphoric acid).

\*Injection volume: 5  $\mu\text{l}$ .

\*Column: C18, 4.6 x 150mm, 5  $\mu\text{m}$ .

\*Detector wave length ( $\lambda$ ): 214 nm.

### **Sample preparation:**

Acacia Nilotica was extracted by grinding the plant by electronic grinder. A 25 grams of finely powder of Acacia was accurately weighed and transferred to 1000 ml amber volumetric flask. About 700 ml of Ultra-pure water was added The powder was passed through amber flask containing 700 ml of ultra-pure water, the solution was mixed thoroughly by magnetic stirrer for 1 hour then completed up to the volume. (Stock 25 mg/ml). At 6 ml of stock was diluted to 100 ml with ultra purified water (concentration 1.5 mg/ml). Sample was filtered by syringe filter 0.45  $\mu\text{m}$ .

### **Specificity:**

**Specificity of the method was evaluated by injection of the standard preparation and sample preparation.**

### **System suitability:**

A 100% of standard solutions was injected 6 times. RSD was calculated.

### **Linearity preparation:**

Five different concentrations 50%, 75%, 100%, 150% and 200% were prepared according to ICH guideline.

**50% Standard preparation:**

A 15 mg of Oxalic Acid working Standard was accurately weighed and then transferred to 100 ml volumetric flask. About 70 ml ultrapure water was added and mixed. The volume Completed to the mark with ultra-pure water. (0.15 mg/ml).

**75% Standard preparation:**

A 22.5 mg of Oxalic Acid working Standard was accurately weighed and then transferred to 100 ml volumetric flask. About 70 ml ultrapure water was added and mixed. The volume Completed to the mark with ultra-pure water. (0.225 mg/ml).

**100% Standard preparation:**

A 30 mg of Oxalic Acid working Standard was accurately weighed and then transferred to 100 ml volumetric flask. About 70 ml ultrapure water was added and mixed. The volume Completed to the mark with ultra-pure water. (0.3 mg/ml).

**150% Standard preparation:**

A 45 mg of O Acid working Standard was accurately weighed and then transferred to 100 ml volumetric flask. About 70 ml ultrapure water was added and mixed. The volume Completed to the mark with ultra-pure water. (0.45 mg/ml).

**200% Standard preparation:**

A 60 mg of Oxalic Acid working Standard was accurately weighed and transferred in 100 ml volumetric flask about 70 ml ultrapure water was added and mixed. The volume Completed to the mark with ultra-pure water. (0.6 mg/ml).

**Repeatability:**

Six replicate samples solutions were Prepared, form the working Standard in same manner of standard preparation (100%).

**Intermediate precision:**

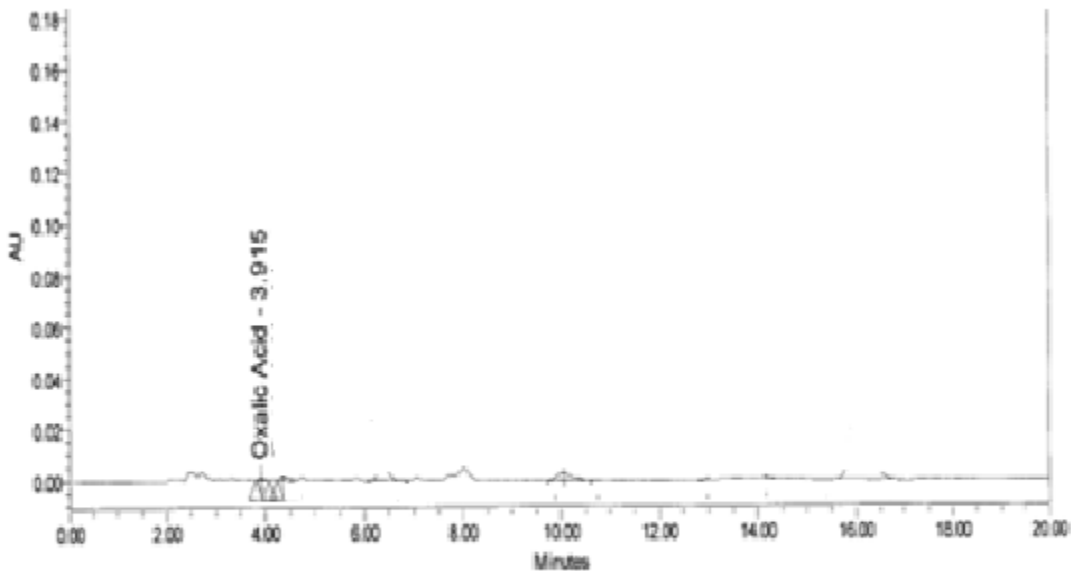
Intermediate precision was evaluated within two different days.

### Accuracy preparations:

At three levels 50%,100% and 150% were prepared in triplicate in the same manner as explained in linearity above.

### Result and discussion:

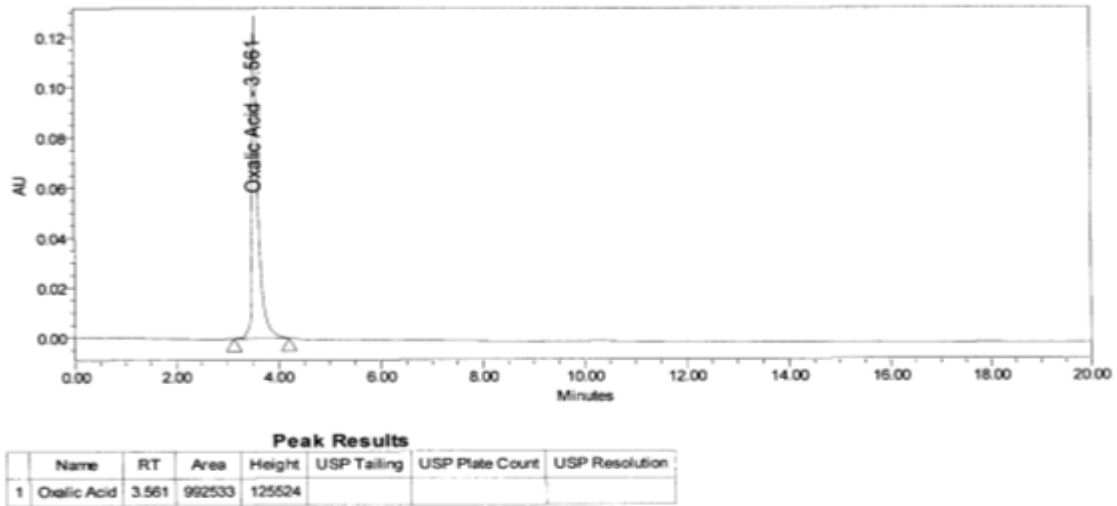
#### Specificity:



Peak Results

Name	RT	Area	Height	USP Tailing	USP Plate Count	USP Resolution
1 Oxalic Acid	3.561	992533	125524			

Sample chromatogram



Standard chromatogram

**System suitability:**

Injection no	Peak area
Injection 1	1114878
Injection 2	1124727
Injection 3	1118896
Injection 4	1121871
Injection 5	1118994
Injection 6	1121281
average	1120107.833
STDV	3343.115279
RSD%	0.2984637

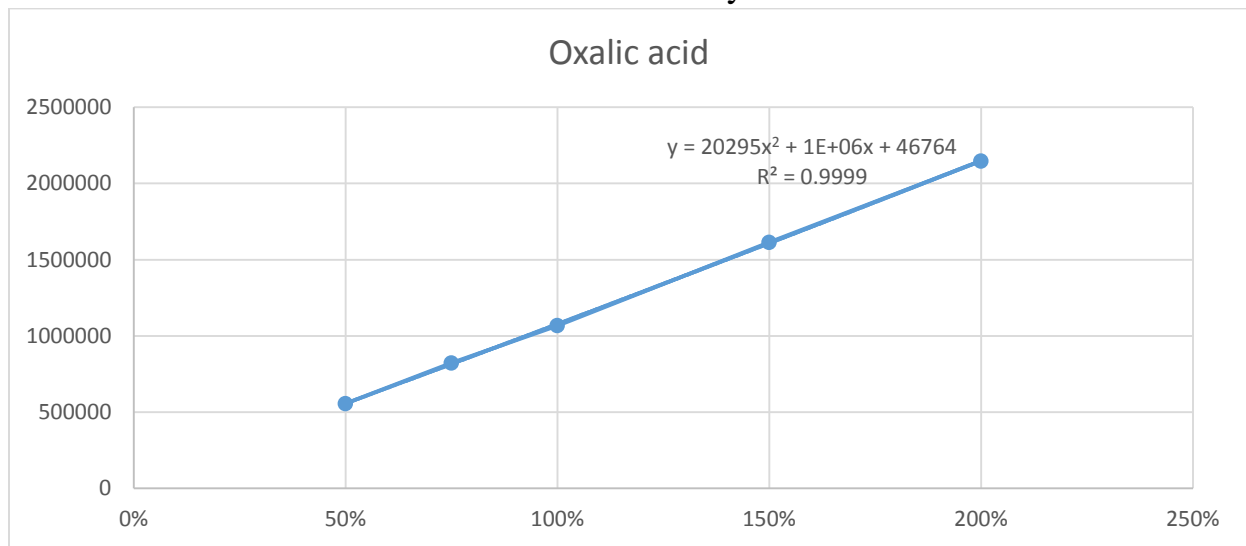
**Method is suitable:**

Acceptance criteria	RSD% $\leq$ 1.5%
RSD%	0.29%

**\* Linearity  
Oxalic Acid:**

Oxalic Acid	50%	75%	100%	150%	200%
1	557849	819887	1069915	1614775	2150019
2	554197	827024	1064722	1613482	2145077
Average	556023	823455.5	1067318.5	1614128.5	2147548
STDV	2582.353965	5046.621097	3672.005515	914.2890681	3494.521713
RSD%	0.46443294	0.612858994	0.344040276	0.056642892	0.162721472

Oxalic Acid linearity table.



**Method is Liner:**

Acceptance criteria	$R2 \geq 0.99$
R2	0.9999

### Repeatability:

Injection No	Peak area of Standard
Injection 1	1063419
Injection 2	1069382
Injection 3	1069209
Injection 4	1070352
Injection 5	1075413
Injection 6	1072169
Av	1069990.667
STDV	3960.951334
RSD%	0.370185597

	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6
	83562684	83792013	83772927	83840691	84139033	83759373
	83614429	83859517	84109433	83940811	84007984	83909271
	83609445	83579161	83936816	83941652	83740416	83929748
Average	83595519	83743564	83939725	83907718	83962478	83866131
STDV	28545.22	146322.8	168271.9	58048.61	203167.4	93020.03
RSD%	0.034147	0.174727	0.200467	0.069181	0.241974	0.110915

	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6
Assay%	101.1311	101.0639	101.2553	101.3214	101.7821	101.9291
average	101.4138181					
STDV	0.356990496					
RSD%	0.352013663					

### Method is Repeatable.

Acceptance criteria	RSD% ≤ 2.00%
RSD%	0.35%

### Intermediate Precision:

	Day 1	Day 2
Assay%	100.04	101.1311314
	98.92640355	101.0638722
	98.68874551	101.2552757
Average	98.37300331	101.1500931
STDV	0.721395303	0.097100409
RSD%	0.7333265	0.095996362

**Method is Precise.**

Acceptance criteria	RSD% ≤ 2.00%	
RSD%	Day 1	0.73%
	Day 2	0.09%

**Accuracy:**

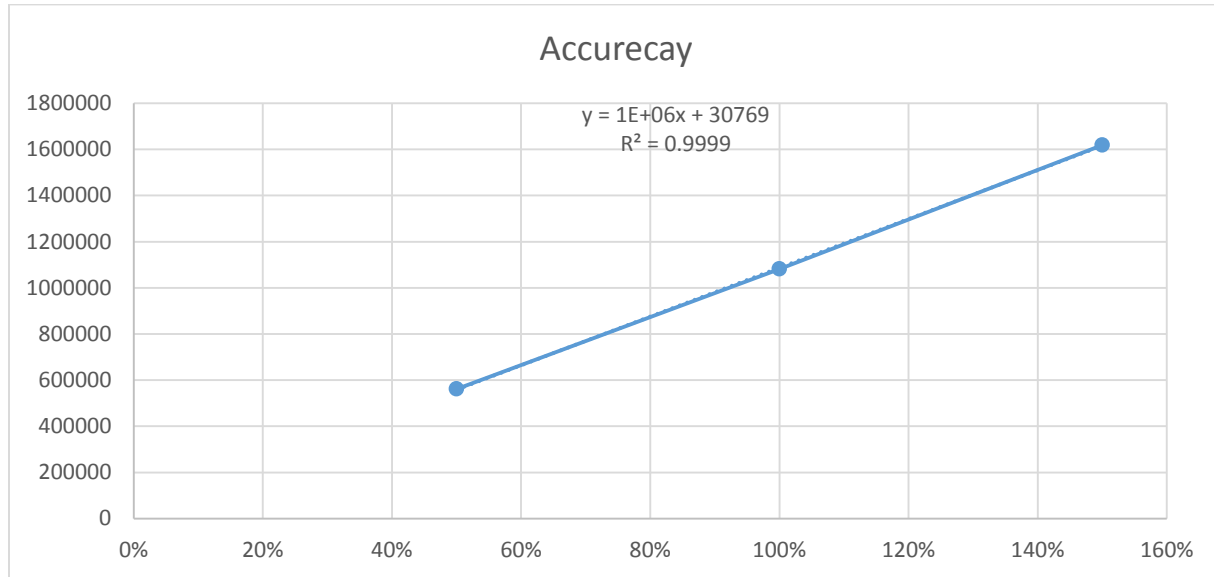
150%			
STD			
Injection 1	1610275		
Injection 2	1637207		
Injection 3	1607769		
average	1618417		
	Test 1	Test 2	Test 3
Injection 1	1614457	1611649	1608345
Injection 2	1627945	1608313	1629466
Injection 3	1629961	1610809	1626644
average	1624121	1610257	1621485
STDV	8429.753	1735.1519	11466.718
RSD%	0.5190348	0.1077562	0.7071739
Recovery	100.35244	99.495804	100.18957

100%	
STD	
Injection 1	1099491
Injection 2	1098012



<b>Injection 3</b>	<b>1091256</b>		
<b>average</b>	<b>1096253</b>		
	<b>Test 1</b>	<b>Test 2</b>	<b>Test 3</b>
<b>Injection 1</b>	<b>1095956</b>	<b>1080882</b>	<b>1079359</b>
<b>Injection 2</b>	<b>1078860</b>	<b>1087335</b>	<b>1079309</b>
<b>Injection 3</b>	<b>1078635</b>	<b>1077418</b>	<b>1076583</b>
<b>average</b>	<b>1084483.7</b>	<b>1081878.3</b>	<b>1078417</b>
<b>STDV</b>	<b>9935.969</b>	<b>5033.0142</b>	<b>1588.4873</b>
<b>RSD%</b>	<b>0.9161935</b>	<b>0.4652107</b>	<b>0.1472981</b>
<b>Recovery</b>	<b>98.926404</b>	<b>98.688746</b>	<b>98.373003</b>

<b>50%</b>			
<b>STD</b>			
<b>Injection 1</b>	<b>573820</b>		
<b>Injection 2</b>	<b>568994</b>		
<b>Injection 3</b>	<b>569336</b>		
<b>average</b>	<b>570716.6667</b>		
	<b>Test 1</b>	<b>Test 2</b>	<b>Test 3</b>
<b>Injection 1</b>	<b>569484</b>	<b>569764</b>	<b>563267</b>
<b>Injection 2</b>	<b>551862</b>	<b>553824</b>	<b>556277</b>
<b>Injection 3</b>	<b>559182</b>	<b>567122</b>	<b>567122</b>
<b>average</b>	<b>560176</b>	<b>563570</b>	<b>562222</b>
<b>STDV</b>	<b>8852.9514</b>	<b>8543.0339</b>	<b>5497.5017</b>
<b>RSD%</b>	<b>1.5803875</b>	<b>1.515878</b>	<b>0.9778169</b>
<b>Recovery</b>	<b>98.153082</b>	<b>98.747773</b>	<b>98.511579</b>



**Method is accurate.**

Acceptance criteria	98% up to 102%
The percent recovery of the spiked placebos should be within 100 ± 2.0% for the average of each set of three weights	99.0%

**Conclusions:**

The method was devolved and validated using HPLC according to ICH guideline. The validated parameters proved that the method is specific, liner, Repeatable, precise and accurate. And I highly recommend to use the method for the analysis of Oxalic acid within chromatographic conditions.

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